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HENRY CHANDLER COWLES

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ERRATA

Vol. LXXXVII

P. 610, last line, for "Agostideae" read "Agrostideae"

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P. 6[illegible] line 8 from bottom, for "(65)" read "(70)"
P. 2[illegible], line 3 from top, for "gametes" read "copulants"
P. 29, line 3 from bottom, for "2^{n-1}" read "2^{n}"
P. 29, line 2 from bottom, for "2^{n}" read "2^{n-1}"
P. 157, last line, for "early transverse" read "early two transverse"
P. 161, line 4 from bottom, for "hypobasal cells" read "hypobasal cell"
P. 205, line 3 from bottom, for "they were" read "there were"
P. 207, legend for fig. 2, for "scale leaf" read "scale leaves"

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THE

BOTANICAL GAZETTE

September 1929

REPRODUCTION IN THALLOPHYTES, WITH SPECIAL REFERENCE TO FUNGI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 393

GEORGE K. K. LINK[1]

Introduction

LAVOISIER, in his *Traité Elémentaire de Chimie* (1793), wrote:

> The impossibility of isolating nomenclature from science and science from nomenclature results from the fact that all science is formed necessarily of three things: the series of facts which constitute the science; the ideas which they call forth; and the words which express them.

Recently four major contributions (**44**, **55**, **59**, **62**) have appeared which consider the problem of sexuality in Thallophytes. One of these, by KNIEP (**55**), is reviewed here with considerable restatement because it contains not only a wealth of factual material, but also statements of ideas in the form of working hypotheses, and a serious attempt to devise simple, precise, and consistent terminologies. This paper, in part, is an attempt to carry this clarification of ideas and terminologies a step further.[2] It is proposed to limit the scope of the concept and term "sexual reproduction," or preferably to dispense entirely with it and its antithetic term "asexual reproduction." The paper also presents briefly the effect of recent genetic analysis on the concepts and methods of mycology and phytopathology.

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[1] I am indebted to Professors E. J. KRAUS and H. E. HAYWARD of the Botany Department for many helpful discussions; and to Professor C. D. BUCK of the Department of Comparative Philology for help in the selection of terms. I am especially indebted to Professor SEWALL WRIGHT of the Department of Zoology for reading the manuscript, for giving me the benefit of his criticism, and for valuable suggestions Thanks are also due my wife, Professor ADELINE DESALE LINK of the Chemistry Department, for helpful criticism and for aid with the literature and the manuscript. However, entire responsibility for the ideas expressed is assumed by me.

[2] A clarification of ideas relative to sexuality is proposed in a paper which has come to my attention since this article was in proof. ALLEN, C. E., Influences determining the appearance of sexual characters. Proc. Int. Congress Plant Sciences. Ithaca, 1926. 1:333–343. 1929 (see footnote 7).

The problem of sexuality in the Thallophytes has been a moot question since the discovery by MÜLLER (61) in 1782 of zygote formation in *Spirogyra*. It has played an important rôle in the motivation and ideology of three of the types of analysis applied to this division of plants. It was the spearhead of the studies of the comparative morphology and life histories of Thallophytes which have continued to this day in unbroken fruitfulness, as a result of the introduction of the technique of pure culture by BREFELD (11), and of experimental physiology by KLEBS (48, 49). It has been one of the main problems with which cytological studies have been occupied and to which some consider they have given a final answer. Latterly, since the rise of genetic studies, the problems of the phenomena associated with and conditioned by sexuality in Thallophytes have received a great deal of attention, with the idea that light might be thrown on genetic hypotheses, because of the phylogenetic position of the division. Consequently we find recent volumes by OEHLKERS (62) and by MORGAN (59), in their discussions of sexuality, sex inheritance, sex determination, and self-sterility, giving consideration to the researches and hypotheses of HARTMANN (43) on relative sexuality in algae, of JOLLOS (45) on relative sexual affinity in algae, and of KNIEP (51, 52, 53), BULLER (15), MOUNCE (60), BRUNSWIK (14), VANDENDRIES (74), and HANNA (40) on pluripolar sexuality of Hymenomycetes. The discussion by OEHLKERS (62) also utilizes recent data from fungi on the problem of the relative rôle of nucleus and plasma in inheritance in plants. In the main the extremely interesting data arising in such studies of Thallophytes have not materially changed the interpretations of geneticists who find it possible to fit the facts into explanations in vogue for higher plants. The attempt to fit the phenomena of relative sexuality of algae and of pluripolar sexuality of fungi into the mold of the self-sterility concept for angiosperms is a case in point.

It is evident from these discussions, and from others reviewed here, that neither the concepts nor the terms sex, sexuality, and sexual reproduction are used in a precise and unequivocal manner. It also is interesting to note that in 1928 MORGAN (59) expressed the same misgivings, voiced by VAUCHER (78) in 1803, relative to sexuality in certain Thallophytes, because no morphological differentiation of male and female elements is present. MORGAN, referring to the so-called phenomenon of pluripolar sexuality in Hymenomycetes, states:

> Here we have the phenomenon of sex exhibited on a grand scale if we interpret the factors involved as sex factors in the conventional sense. . . . . It may seem of doubtful value to identify these factors with sex factors which conventionally at least apply to somatic differences in dioecious types or to those with separate sexes. It is true that amongst these differences are those concerned with producing eggs and sperms whose main function is to unite with each other, but, as generally understood, these functions are less conspicuous than those appertaining to the bodily constitution of male and female individuals.

Rôle of sexuality concept; genetic studies

Before considering in detail the confusion in ideas and terms relative to sexuality in Thallophytes, a great deal of which is inherent in the complexity, diversity, and intergradations of this, like all other biological phenomena, the significance of the concept of sexuality and the research which it has inspired and directed will be briefly discussed. The facts unearthed in the course of the studies of sexuality of Thallophytes and the ideas they have engendered have directly and indirectly led to significant theoretical and practical results, not only with reference to Thallophytes, but also to higher plants. Thus the foundations laid by KLEBS (48, 49) in his studies of the influence of environmental factors in the life histories of Thallophytes have been used by KRAUS and his associates and students (56) to arrive at facts and ideas whose application has profoundly modified horticultural and floricultural research and practices (18) in the United States. A comparative morphology of fungi by GÄUMANN (37) and GÄUMANN and DODGE (38), which is rational and does not lose itself in a morass of detail, has resulted from the utilization of the concept of alternation of nuclear phase as a *leitmotif*. The most far-reaching effects, however, especially since the rise of genetic analysis, have been experienced in the fields of mycology and phytopathology. The discovery of homothallism (monoecism) and heterothallism (dioecism) as well as of the possibility of hybridization in the Mucorales by BLAKESLEE (6), and the discovery by BURGEFF (16) that "sex" characters are inherited independently like Mendelian characters in these fungi, have led to theoretical and practical consequences whose significance is just beginning to be exploited on a comprehensive scale.[3] As a result of studies for which these pointed the way, it is now realized that pure culture, in the sense of starting with a single spore or cell, the corner-stone of bacteriology, as well as much of mycology and algology, is not adequate in the study of Thallophytes, because heterothallic forms do not come to full expression. On the other hand, it is inadequate also, as pointed out by BRIERLEY (12, 13), because a single spore or cell may be heterozygotic. Thus, a smut spore and a sporidium from the promycelium of that spore may be quite different genetically. Consequently hybridization and other methods of the geneticist must become part of the technique of the algologist, mycologist, and phytopathologist, so that they may segregate the genotypes and effect new combinations. Tools have thereby been placed in the hands of the latter for attacking the problem of specialization and of biologic species of parasites, and to determine whether the phenomena involve: (1) modifications (Modificationen, Paravariation, in the sense of BAUR 3); Dauer-modificationen, in the sense of JOLLOS (variations which may persist for a long time but which are eliminated in caryogamy and reduction); (2) combinations (Kombinationen, Mixovariationen in the sense of BAUR 3); or (3) mutations (Mutationen, Idiovarianten in the sense of BAUR, that is, variations which persist through caryogamy and reduction and represent true genotypes).

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[3] Heterothallism has been experimentally indicated or demonstrated for the following Phycomycetes and Ascomycetes: *Dictyuchus monosporus* (COUCH 25); *Phytophthora faberi* (ASHBY 1 and GADD 36); certain Laboulbeniales (THAXTER 73); *Glomerella cingulata* (EDGERTON 35); *Ascobolus magnificus* (DODGE 32); *Penicillium luteum* (DERX 29); *Ascobolus carbonarius* (BETTS 5); certain Taphrinales (WIEBEN 80); and *Neurospora sitophila* and *N. crassa* (SHEAR and DODGE 68). The Basidiomycetes have proved an especially rich field for the application of the principles discovered by BLAKESLEE and BURGEFF. BENSAUDE (4) discovered heterothallism for Hymenomycetes in 1918; KNIEP (50) reported the same for Ustilaginales in 1919, and later for Tremellales (55); and CRAIGIE (26, 27, 28) discovered it for Uredinales in 1927. KNIEP (51, 52, 53) reported that copulation in Hymenomycetes is controlled by Mendelian factors; and BAUCH (2) reported the demonstration of secondary (physiological) sex characters for *Ustilago violacea*. KNIEP first (50) reported success in hybridization of smuts, not only between biologic races of *Ustilago violacea*, but also between species, such as *U. nuda*, *U. tritici*, *U. hordei*, and *U. bromivora* (54).

Goldschmidt (39), in a critical research, has applied himself to this question, in the hope of throwing light on the problem of specialization and on the more general problem of evolution of species. The research was designed to test critically the validity of the various hypotheses that have been advanced to account for the origin and nature of physiologic species. Biologic species have been taken to have their origin in (1) active adaptation of the parasite under formative influence of the host; (2) variation, induced by host influences (Dauer-modificationen); and (3) mutation and selection which lead to genotypically different races.

Goldschmidt used the biologic species of *Ustilago violacea* for this analysis. The reactions of the strains of the biologic species were tested against test stocks (A and a) to determine their "sexual reaction." With these tested strains, hybridization was carried out, and eleven hybrids of biologic races of *U. violacea* and six crosses between biologic races of this fungus and other species of *Ustilago* were tested for pathogenicity. Discouragingly low incidences of infection were obtained. Of the twenty-five control tests with mixtures of sporidia of the opposite sex on their hosts, infection occurred in only six hosts. Out of the eleven expected hybrids, six were recovered in appreciable quantity. The reason for the low percentage of infection was not determined. Possibly immunity and virulence phenomena, or unfavorable environmental factors were involved. At times the pathogenicity of the hybrids was different from that of the parents. Thus one hybrid did not inhibit pistil formation in *Melandrium album* (♀) as did its parents. The promycelia of parents and hybrids were studied critically, since no difference was detected between spores and sporidia, the hybrid promycelia as a rule being larger than those of the parents. One hybrid promycelium exhibited the morphological characteristics of one parent. Another hybrid gave irregular "wild" germination. Back crosses of hybrids and both parents were made, infection tests were carried out with these, and studies made of spore germination in the recovered progeny.

Goldschmidt concluded that in the biologic races of *U. violacea:* (1) Dauer-modification occurred and that one of these involved plasmatic alteration (possibly diseased plasma); (2) a mutant was found which probably is of plasmatic origin; and (3) specialization is independent of the plasma of the fungus, and the biologic races of *Silene saxifraga* and *Melandrium album* differ by one Mendelian gene which determines specialization. Haploid infection does not occur, but infection does occur when only one nucleus of the dicaryophase is specialized for the host. Dominance in the dicaryophase in the hybrid race (*Silene saxifraga* × *Melandrium album*) was not manifested, as was reported for *Schizophyllum* by ZATTLER (82). It is also contended that the recovery from one hybrid of the characters of both parents proves that the brand-spore contains factors for these, and that since fusion of sporidia is the only place where they can possibly come together, copulation of sporidia is a sex act and that the sporidia physiologically are gametes. LAIBACH (58) has recently contended that sporidial fusion in the smuts is the functional equivalent of cell fusions in germinating conidia of Ascomycetes. BOSS (10) contends that it should not be considered a copulation but a fusion.

This contribution by GOLDSCHMIDT promises to mark a new era in the study of specialization and of physiologic races in fungi. STAKMAN (70) has recently reviewed this subject, and it is evident that heretofore ideas relative to specialization have been based on infection experiments, or cultural criteria of a morphological or physicochemical nature, and on inferences based on a few successful hybridizations and so-called mutations in fungi.

This study also points an additional way to analysis of mutation which is reported to occur abundantly in the fungi (70), and for which a summary was given by CHODAT (20). The most recent reports are from CHRISTENSEN and STAKMAN (21), CHRISTENSEN (22), and from CHIH TU (19). These mutations have been interpreted as genotypes by some investigators (65), even though no genetic analysis was made. That true mutations occur in fungi has been amply proved by the researches of KNIEP (53, 55), BRUNSWIK (14), VANDENDRIES (75, 76, 77), and HANNA (40) for Hymenomycetes. In fact, these studies indicate that geographic races may be due to genetic mutations and production of multiple allelomorphs.

The studies of HARDER (42) throw light, not only on the relative rôle of nucleus and cytoplasma in heredity, but possibly also on "mutations" and "saltations" reported in fungi. HARDER attacked this problem directly by removing one of the paired nuclei from clamps of *Schizophyllum commune* and of *Pholiota mutabilis* with a micro-manipulator, and then observing the behavior of the operated cells. It was demonstrated that the operated cells withstood the shock, and that sexuality, clamp and fruiting body formation, and other characters are controlled by the nuclei. Hold-over influences of the ejected nuclei were noted in the formation of pseudo-clamps for some time after the operation. The habit of *P. mutabilis*, at least, was found to be controlled by the plasma to a great extent. A great number of the "mutations," "saltations," and "fluctuations" reported for fungi are changes in the habit of the organisms under study. In the light of HARDER'S study these may be temporary or permanent changes due to cytoplasmic conditions. Genetic studies are necessary before a final interpretation can be attempted for most of such reports of "mutations." Changes in habit similar to those reported as "mutations" have been induced by ultra-violet irradiation of fungi by STEVENS (71), and by RAMSEY and BAILEY[4] from the Cooperative Laboratory of this Department and of the United States Department of Agriculture.

The recent reports by DICKINSON (30, 31) for *U. levis*, by GOLDSCHMIDT (39) for *U. violacea*, and the less conclusive report by STAKMAN and CHRISTENSEN (69) and the convincing report of HANNA (41) for *U. zeae*, substantiate the earlier reports of ZILLIG (83) for *U. violacea* and of KNIEP (54) for *U. avenae*, to the effect that in some fungi only the dicaryophase is pathogenic. These, together with the report of GOLDSCHMIDT (39) that pathogenicity for a definite host is determined by a genetic factor, are destined to influence the concepts of virulence and of susceptibility and resistance. It has been apparent for some time that these concepts need recasting.[5] Success in hybridizing species of fungi and obtaining hybrids that ran the full course of their life history is reported by DODGE (33) for the Ascomycete *Neurospora*. This is significant in the light of previous reports of success in obtaining zygotes in Phycomycetes, and hybrids in Ascomycetes and Basidiomycetes, most of which, however, failed to germinate or to complete the life cycle.

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[4] In press. BOT. GAZ. 89:

[5] SHARP, working in this laboratory, discovered that variation in virulence in *Bact. phaseoli sojense* is correlated with a rough-smooth variation and with variation in other morphological and physiological characters. SHARP, C. G., Virulence, serological, and other physiological studies of *Bacterium flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. BOT. GAZ. 83:113–144. 1927.

Application of the principle that certain Hymenomycetes are heterothallic has been used by L. F. BUTLER of the Cooperative Laboratory of this department and of the United States Department of Agriculture, in solving the tangle of the identity of certain rots of apples, from which "sterile" mycelia were quite consistently isolated by him.[6]

In view of the practical and theoretical applications of ideas based on them, it is not surprising that the phenomena of sex in Thallophytes, to which KNIEP'S entire volume (55) is devoted, have aroused such interest. In another volume, HARTMANN (44) considers these phenomena as a part of his general discussion of reproduction, fertilization, and sexuality in the chapter on "Form-wechsel." OEHLKERS (62) gives them much attention in his discussion of modern genetics as applied to plants, and MORGAN (59) takes up these topics incidentally in his discussion of sex determination.

Causality of sexuality

HARTMANN attempts a causal analysis of sex phenomena, and follows BÜTSCHLI and SCHAUDINN in postulating that sexuality is one of the characteristics of fertilization and that it is unconditionally associated with it. He uses the term sexuality as the antithesis of male and female, which consists in a "sexual difference," at least a physiological one. This difference is taken to involve sex substances (Geschlechtssubstanzen) and to be probably nuclear. In formulating his theory of sexuality he admittedly follows CORRENS (23, 24), postulating that every sexually differentiated individual and germ cell possesses complete male and female potentialities (Potenzen). Preponderance of one of these complexes, due to inhibition of the other, produces a male or female tendency (Tendenz) in the cell. HARTMANN postulates that varying male and female tendencies may be conditioned by different quantitative mixtures of the true sex substances, and when the requisite sexual tension (gradient) has been realized between two gametes, they may fuse (relative sexuality). Varying tendency (with equal potentialities) may be induced by various external influences or internal developmental factors at different stages of development of the individual (phenotypic sex distribution and determination); or by special hereditary factors, sex determiners (realizators, differentiators), which are not the only factors, however, so that reduction division and fertilization (genotypic determination) play the decisive rôle in the distribution and determination of sex.

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[6] In press. Jour. Agric. Res. 39:

HARTMANN (43) contends that his experiments with *Ectocarpus siliculosus* furnish the necessary facts to give support to his hypothesis. In this alga isomorphic gametes are involved that behave anisogamously in copulation, which HARTMANN takes to be a criterion of sexual differentiation. HARTMANN'S material was strictly dioecious. The gametes designated as female come to rest sooner than the male. The rate and frequency of copulation between gametes of various plants varied so that some males were designated as strong or weak males, while correspondingly on the basis of rate of settling the female gametes were designated as strong or weak females. Some plants produced gametes which reacted equally strongly as males or females. The strong males copulated not only with all females, but also with weak males, while strong females copulated with all males and with weak females. These phenomena he designated as relative sexuality.

The theory of sex substances, which was cautiously advocated by BLAKESLEE (7) in 1920, and the theory of direct correlation between maleness and femaleness and quantitative differences in qualitatively different male and female substances as developed by HARTMANN (44), are interesting and simple ones. Their experimental analysis may carry one into what WILSON (81) referred to as "an inaccessible field of inquiry," and involves the isolation of the so-called "sex substances." Possibly no pair of single substances is involved, but rather states or conditions which may be either physical or chemical, or both, and different in various plants. Modern genetic interpretations would suggest that a condition or substance complex, rather than two single substances (sex substances) is involved. Although no data are available on the isolation of fundamental sex substances in the Thallophytes, an extensive literature deals with the physiological, biochemical, and physico-chemical characteristics of sexual individuals. While it is not contended that they cause the sexes, these differences are reported to characterize them. In organisms in general, the male sex is taken to be characterized by an activity (motility) metabolism, and the female by a passivity (storage) metabolism.

Among fungi, especially the Mucors have been extensively used in such studies because they are taken to represent sex in its simplest forms. Beginning with KORPATSCHEWSKA (55a), the Mucors have been studied to determine whether the plus and minus strains show physiological characteristics other than fusion; and latterly possible biochemical and physico-chemical characteristics have been sought. The literature of this topic has recently been summarized by SCHOPFER (67). Following KORPATSCHEWSKA (55a), contradictory reports have appeared relative to the characteristic abilities of mycelia of plus and minus strains in the utilization of given nutrients at definite temperatures. Recently SCHOPFER (67) carried on an extensive piece of research with *Mucor hiemalis*, and reports that consistent differences, such as rate of increase in diameter, absorption, reaction toward toxins, presence of carotin, effect on bacteria, and possibly serological specificity, exist between the plus and minus strains. He also reports that the megacopulation-branch is characterized by an abundance of fat. BURGEFF (16) reports that characteristics which are considered sexual in Phycomyces are separated from the sexes in meiosis. ORBAN (62a), on the other hand, reports that linkage was maintained through meiosis. In many studies of the characters of plus and minus strains this essential test has not been applied to the material, with resultant loss in value.

SATINA and BLAKESLEE (64, 65, 66) report for the Mucors, which show not only dimorphism (8, 9) but also all gradations between extreme plus and minus strains, that quantitative biochemical differences exist between plus and minus individuals. They used among others the Manoilow test, by which relative oxidation and reduction are measured, and report that the plus strains are relatively more reducing while the minus are more oxidizing. JOYET-LAVERGNE (47) was unable to demonstrate a similar difference in the oxidation-reduction potential (rH) for the plus and minus strains of *Coprinus* spp. supplied him by VANDENDRIES, but reports success with the micro- and megagametangia of *Fucus vesiculosus* and *F. serratus*.

There is no agreement even among biochemists as to the reliability of the Manoilow and similar methods. BURGEFF and SEYBOLD (17), who tested among other plants the extract of *Phycomyces blakesleeanus* by the same methods, conclude that they did not obtain consistent differences, and after running careful tests of the methods themselves, question their validity.

Even if the methods are valid, and the reports of positive results are substantiated, these experiments do not establish that qualitatively different sex substances exist in the sense of HARTMANN'S and BLAKESLEE'S earlier hypotheses. At best, these are secondary sex characters, the accompaniments or results, rather than the causes of sexual differentiation. It would be rash, however, to assume that further research might not establish the existence of specific qualitatively different sex substances.

The fundamental assumption made by HARTMANN, that copulation is conditioned by some sort of *difference* in the copulants which sets up a tension relieved at time of fusion, is grounded in the general dictum that likes repel and unlikes attract. It has an analogy in certain chemical reactions in which a balancing of plus and minus charges is concerned. However, it is not necessary to assume that fusion of copulants depends on differences. If we turn again to chemistry for analogy, we find instances of union between identical entities, as for example the union of oxygen or fluorine atoms to form molecular oxygen or fluorine. The assumption here made is that two identical unstable atoms pass into a stable state by sharing common electrons. This seems to be the method of combination of the carbon atom which plays an important and characteristic rôle in life phenomena. Attraction of likes is also indicated by chromosome orientation and conjugation, as well as by gene arrangements on homologous chromosomes. It seems to be a factor in successful hybridization between closely related organisms, and in the failure of attempted hybridization of distantly related or unrelated organisms.

JOLLOS' (45) results with *Dasycladus clavaeformis*, in which a change in relative sexual reaction was obtained by placing gametes of one sex into a filtrate of a suspension of gametes of the opposite sex, led him to conclude that he had not altered the sexual tendency but rather the sexual affinity of the gametes. HARTMANN apparently does not consider this as critical an experiment as do others; at least he does not discuss its implications.

Kniep's interpretation of sexuality in Thallophytes

KNIEP'S book had its origin in notes which he made in the course of his extended studies and those of his students on conjugate algae, brown algae, Hymenomycetes, and other Basidiomycetes. The volume, which is permeated with a genetic atmosphere, is a veritable mine of facts, oriented by clear ideas, which in turn are expressed in terms chosen with discrimination so that mere verbalism is avoided. The treatment of the complex detailed subject matter is characterized by sharp differentiation between fact and hypothesis; data are critically reviewed; gaps are pointed out; working hypotheses for bridging and filling them are suggested. The treatment attains unity because the problems of sex determination and physiological sex differentiation are kept in the foreground, and constitute the real theme of the book. The high points in the book are the discussions of studies by KNIEP and his students. These indicate by contrast what must still be done before a general theory can be attempted for sexuality of Thallophytes. The volume is well illustrated, and diagrams are used extensively to clarify the discussions of alternation of nuclear phases and of generations. KNIFP distinguishes between alternation of generations and alternation of nuclear phase, and subscribes to the view that the concept generation is not applicable when a gamete or a zygote would have to constitute a generation. He does not subscribe to the contention that the concept alternation of generations can be used only in a dual sense, but is willing to look upon each individualized body, or each body which produces a distinct spore type, as a generation. Thus the diplo-biontic Floridiae are considered by him to possess two nuclear phases and three generations, gametophyte, carposporophyte, and tetrasporophyte. He states, however, that it is debatable whether the aecium and the promycelium in *Puccinia graminis* should be considered as generations, and chooses not to consider the promycelium a separate generation because he does not care to be compelled to consider an ascospore or the basidiospore of Autobasidiomycetes a separate gener ation. Naturally alternation of nuclear phase and of morphological form are not always correlated. KNIEP takes the stand that it is misleading to apply the term "spore" to gametes or to the immediate fusion products. There is no attempt to discuss purely phylogenetic problems. KNIEP incidentally states that he considers the ascus and basidium as homologous structures, the latter being derived from the former. Autobasidiomycetes are considered the simplest Basidiomycetes, with their holobasidia, and from them those with septobasidia are taken to be derived. The brand-spores of the Ustilaginales are considered to be basidia which become sclerotic in youth (sclero-basidia). KNIEP is quite confident that future research will prove conclusively that CLAUSEN'S findings and interpretations of the nuclear phenomena in the Ascomycetes are correct, and that there is no double fusion and reduction. The main body of the book is prefaced by an interesting historical survey of the vicissitudes of the sexuality concept for Thallophytes, and concluded with a masterly summary in which the author clearly and unequivocally defines his concepts and his nomenclature, which draws freely upon previous nomenclatures, but is a simplification of current terminologies. Some new terms are introduced. He also states his views on the theories of relative sexuality, pluripolar sexuality, and self-sterility.

KNIEP does not define the concepts sex, sexuality, or sexual reproduction, but points out that the last covers a series of individual processes which in the main are characterized as follows: The series begins with differentiation and maturation of elements, the copulants, which come together by chemotropism, chemotaxis, or chemomorphism, depending upon their constitution. The first step of the sex act is cytogamy, which is followed at once, or after a more or less protracted dicaryophase, by the second step, caryogamy. Caryogamy is followed by chromosome conjugation, and then reduction division brings the series to a close.

KNIEP considers that caryogamy and reduction division are the fundamental features of sexual reproduction, and that there are no other general criteria ("weitere allgemeine Kriterien der sexuellen Fortpflanzung gibt es nicht"). In making his point that copulation of "specially differentiated, uninucleate cells which fuse to form zygotes," the gametes, is not the essential criterion of sexual reproduction, KNIEP points out that copulation is not restricted to gametes, gametogamy, but that it also occurs between gametangia, gametangiogamy, or between somatic cells, somatogamy. The common features of all of these, however, are caryogamy and reduction division. Granted this point, the nuclear phenomena in *Hypochnus terrestris* and *Puccinia arenariae* are sex phenomena of an autogamous type. Intergrades occur. Thus in *Saprolegnia* and *Collema* there are combinations of gametogamy and gametangiogamy; in *Ascobulus carbonarius* there is a combination of gametangiogamy and somatogamy. Gametes may occur as planogametes or aplanogametes. The copulants (gametes, gametangia, somatic cells) may be isomorphic or anisomorphic. Identical behavior of isomorphic copulants constitutes isogamy. KNIEP questions whether there is true isogamy from the physiological point of view. (As pointed out before, it is not necessary to assume that copulation is determined by differences, and if this be true, there is no reason for assuming that real isogamy does not exist.) According to KNIEP, anisogamy in its simplest form is characterized by divergent behavior of the isomorphic copulants while oogamy is the most advanced type of gametic anisogamy. Both isogamy and anisogamy may occur in gametogamy, gametangiogamy, and somatogamy. Isogamy and anisogamy are concepts which offhand apply to the phenotypic aspect of copulants. Differentiation of copulants into male and female may be determined by caryogamy and reduction division (genotypic), or in the course of development (non-genotypic). Haplogametophytic Thallophytes are grouped by KNIEP on the basis of the character of their copulants as follows: (1) Phenotypically and genotypically alike; examples among fungi are *Olpidium viciae*, *Sporidinia grandis*, and *Schizosaccharomyces octosporus*. (2) Phenotypically alike but genotypically different; examples among fungi are *Mucor mucedo*, *Phycomyces nitens*, *Taphrina epiphylla*, *Aleurodiscus polygonius*, *Schizophyllum commune*, *Coprinus fimetarius*, *Ustilago violacea*, and probably *Puccinia helianthi*. (3) Phenotypically different but genotypically alike; examples among fungi are *Monoblepharis*, most Saprolegniaceae, *Dipodascus*, anisogamous yeasts, *Polystigma rubrum*, *Pyronema confluens* and *P. domesticum*, *Ascodesmis nigricans*, and all monoecious Laboulbeniales. (4) Phenotypically and genotypically different; examples among fungi are *Dictyuchus monosporus*, *Pericystis apis*, *Ascobolus magnificus*, and *Dioicomyces*.

KNIEP here uses the term "phenotypic" in the sense of appearance only (phaenotypisches Bild). It is debatable whether the restricted use of the term is a happy choice. Phenotypic is used by many to mean anything in the nature of a character, be it morphological or physiological. It is a question whether copulants can be strictly alike phenotypically in this sense when they are genotypically different. Type 2 of KNIEP's list may therefore be merely a special case of type 4. Certainly *Taphrina epiphylla* shows phenotypic differences in the behavior of its copulating conidia, and *Ustilago violacea* in the physiologic behavior of its sporidia in various culture media.

Diplogametophytic forms (occurring only among the algae) show a diversity of sexual reproduction, and for the dioecious forms the mechanism of sex determination is assumed to be like that of some phanerogams, that is, a combination of homogamety and heterogamety. Because of the use by geneticists of the term heterogamety in contrast to homogamety, KNIEP adopts the term anisogamy in preference to heterogamy which has generally been used in contrast to isogamy. KNIEP is not entirely consistent in the use of the term "gametophyte." He insists on the sharp differentiation between gamete, gametangium, and soma, which seems to make for precision and clarity of concepts and terms, yet he takes the liberty with the term gametophyte of applying it to any soma which bears gametangia, or to somatic cells functioning as copulants, as is the case in most fungi. The term copulantophyte could be used to designate those somas which bear copulants other than true gametes. If the soma bearing any type of copulant is to be referred to as a gametophyte, then the clarity and precision gained by distinguishing between gamete, gametangium, and soma as copulants are entirely lost.

KNIEP also suggests the following classification of sexual reproduction based on derivation of the copulants: (1) autogamy: obligatory copulation of the elements of one individual which may be (a) cytogamous (cells of the same individual) or (b) acytogamous (nuclei of the same cell); and (2) xenogamy, the copulants being derived from different individuals.

A discussion of parthenogamy (in Ascomycetes) leads to the question whether copulation takes place between two male or two female copulants. At this point KNIEP takes up HARTMANN'S concept of relative sexuality and the phenomena of pluripolar sexuality in Hymenomycetes. Originally KNIEP had designated as sex factors those which control matings of mycelia and which, he found, behaved like Mendelian factors. He assumed the presence of like factors prevented matings. In the light of criticisms of this use of the concept and term "sex factors," he adopts the suggestion of OEHLKERS (62) to assume that copulation factors are involved, and thereby gather the phenomena of relative sexuality, relative sexual affinity, and pluripolar sexuality under the one concept "copulation factors." In accepting this suggestion, KNIEP now refers to the phenomena in Hymenomycetes as a "pluripolarity of copulation determining factors." The genes occur as multiple allelomorphs, and in the tetrapolar type two pairs of such allelomorphs, located on two pairs of chromosomes, are involved. The fundamental assumption is made that like genes prevent copulation. His argument in favor of copulation factors is as follows: Strictly speaking, the conditions determining copulation of isomorphic copulants which are genotypically different must have their basis in differences of the physiological structure. These cannot be assumed to be anything but copulation factors, which at first need not be genes. Copulation therefore involves a minimum of two different factors, which however are not the same ones that elicit sex differences (sex factors). Later, these two sets of factors may become linked, thus giving rise to true anisogamy (anisogamety). Consequently, for *Ectocarpus siliculosus*, he postulates that the linkage of copulation-determining factors and of sex-differentiating factors is not rigid. He rejects HARTMANN'S hypothesis of sex factors, admitting, however, that it is a simple and consistent one.

PRELL (63), BRUNSWIK (14), VANDENDRIES (75), VON WETTSTEIN (79), OEHLKERS (62), JONES (46), and MORGAN (59) have suggested that the phenomena of pluripolar sexuality in Hymenomycetes may be a matter of self-sterility factors, and OEHLKERS has pointed out that copulation factors could just as well be called sterility factors, and thus one concept serve for all the phenomena in algae, fungi, and flowering plants. KNIEP rejects these suggestions. He contends that the fundamental assumption necessary in this hypothesis, that is, that all haplonts of isogamous Thallophytes are monoecious in that they carry potentialities for both sexes, and that therefore pluripolarity in Hymenomycetes is merely an expression of self-sterility as in monoecious flowering plants, is unwarranted. He contends that there is no ground whatsoever for assuming that isogamous Thallophytes, even monoecious Hymenomycetes, possess sex potentialities, pointing out that he believes that he is justified in applying the extended concept "monoecious" to them because there is no genotypic or phenotypic segregation of the copulation factors.

At this point KNIEP seems to depart from his otherwise rigorously logical analysis. If he assumes that in isogamous forms only copulation factors are involved, and that these are not in themselves sex factors nor linked with them, then the reproduction following copulation of these copulants, it seems, should not be considered an instance of sexual reproduction. This, however, leads one to the necessity of denying the validity of KNIEP'S fundamental assumption, that is, that the only essential criteria of sexual reproduction are caryogamy and reduction.

He contends further that self-sterility phenomena in *Nicotiana* and *Veronica* are not identical with the phenomena of pluripolar sexuality, for in the former reactions between diploid and haploid somas are the deciding factors, union of the haploid somas taking place if given an opportunity; whereas in the fungi it is an incompatibility between haploid elements that is involved. There may be merit in this objection. On the other hand, if we assume that something like an immunological reaction is involved, then it is not unreasonable to assume further that such reactions may take place between diploid cells, between haploid cells, and between diploid and haploid cells, so long as each pair contains factors whose balance leads to a production of the same state or substance. Certainly it is not necessary to postulate that the same things are involved in all of these reactions; one thing may be involved in fungi, another in flowering plants.

VANDENDRIES (**76, 77**), on the basis of extensive and exceedingly laborious investigations on Hymenomycetes, especially *Coprinus* *micaceus*, has drawn very significant conclusions from his remarkable results. These results are in part identical with those reported by KNIEP (55). VANDENDRIES proposes a working hypothesis to explain the dihybrid or modified dihybrid copulation within the mycelia derived from one sporophore of *C. micaceus* as well as the essentially perfect fertility, on the one hand, and the essentially perfect sterility on the other, between mycelia of spores derived from sporophores of different localities, both near and distant.

He accepts KNIEP'S hypothesis that copulation between mycelia derived from spores of the same sporophore, and greater fertility between spores of different sporophores are due to sexual allelomorphic genes (KNIEP'S recent copulation-determining factors), and that these phenomena are the basis of the so-called geographic races of Hymenomycetes. KNIEP (55) had expressed the opinion that this sterility between spores of sporophores from different localities might be due to factors which determine sterility between closely related species, and that it might depend upon excessive differences of the copulation-determining genes, or upon secondary factors. VANDENDRIES independently developed the idea that factors are operative in sterility akin to those which determine sterility between species, but he postulates that these are distinct factors, designating them as "dominant genes" in contradistinction to the sexual genes of KNIEP, to which he refers as "Kniepian genes." These dominant genes are taken to be subject to qualitative and quantitative variation which leads to mutation. The presence of the same dominant gene guarantees the possibility of copulation provided that the proper Kniepian genes are present. The dominant genes mutate independently of the Kniepian genes, and thus it is possible for sporophores to arise, none of whose spores will copulate with those of a sporophore from another region. The quantitative and qualitative differences permit intergrades, so that the mycelia of spores of an intermediate sporophore may copulate with those of spores of two other sporophores which have entirely different dominant genes between each other. This hypothesis of dominant factors in addition to Kniepian genes has much to commend it. It is in line with current genetical thought on the extremely complicated problems of sterility and fertility of organisms which JONES has recently summarized (46). Certainly it seems possible that in fungi different sets of factors may determine compatibility between spores of different species, of different races, of different sporophores, and of the same sporophore if different and even independent sets of factors, which have been designated as fertility, sterility, compatibility, and incompatibility factors, determine ability of pollen tubes to grow, and selection between gametes of different individuals, and between gametes of the same individual.

VANDENDRIES points out that he considers all sporophores with the same dominant gene as constituting a "geographic race," irrespective of whether they show typical dihybrid copulation (in *C. micaceus*) or perfect fertility; and those with a different dominant gene, and therefore showing complete sterility, as another geographic race. According to KNIEP, sporophores which are perfectly fertile or sterile with nearby or distant sporophores belong to different geographic races. It does not seem advisable to designate these as geographic races when the real criterion of both authors is copulation or non-copulation. Biologic races would be a preferable term.

The concepts monoecious and dioecious are extended by KNIEP to isogamous forms. Those which produce copulable copulants in one individual are designated as monoecious, while those in which copulable copulants must come from different individuals are considered dioecious. The terms have only a phenotypic significance. It is debatable whether it is wise to extend terms which have attained and held quite definite and different meanings for a long time, especially when it is questionable whether sex is at all involved.

As used by KNIEP, the concept apomixis includes the following phenomena: (1) gametic apomixis (gametic parthenogenesis and ephebogenesis); (2) gametangial apomixis, both of which may occur in isogamous and anisogamous forms; and (3) somatic apomixis.

KNIEP does not attempt to formulate a general theory of sexuality on the basis of the phenomena noted in Thallophytes. He questions whether sexuality can be causally explained on a single principle as attempted by HARTMANN. He is inclined to postulate that the polyphyletic origin of sexuality in the Thallophytes indicates that the physiological processes which lead to fertilization may be very diverse, and that a unifying explanation may lie in ecological considerations. Since amphimixis undoubtedly leads to increased variability, provided different genotypes enter into the zygote, which is not the case in "haplo-monoecious" organisms, KNIEP queries whether alternation of nuclear phase may not be of significance for mutability (für Mutabilität). It might be better to say that it may prove that alternation of nuclear phase as such may play a rôle in association with mutability. Mutation might occur in nuclei of monoecious forms if a rather long interval elapsed between pairing and fusion of nuclei.

Proposed clarification of concepts and terms relative to reproduction and sexuality

As pointed out before, there is confusion in the sex terminology. The difficulty is in part due to the fact that the same concepts and terms are applied to different things and situations, and that they are used in a specific or restricted, as well as in a general sense, quite indiscriminately. Another source of trouble lies in the fact that biological phenomena successfully resist classification. It is being assumed in the following discussion that the concept and term sex is the fundamental original one, and that the concepts sexual and sexuality are derived from it. In other words, sexual means "of or pertaining to sex" and sexuality "the character of sex." Sex is here assumed to mean *differentiation of a specific sort* which developed in association with fusion of copulants and zygote nutrition, protection, and development; that is, differentiation of individuals who themselves or whose individualized products are characterized on the one hand by activity, and on the other by passivity (storage metabolism), and generally also by distinctive size or form or both.[7] This interpretation is in harmony with common usage. The concepts sex, male, female, are probably originally based on phenotypic bipolar differentiation of diplonts, as seen in man and the higher animals, that is, animals without alternation of generations, and possibly (certainly to a much more limited extent) on phenotypic differentiation of the sporophytic generation of diplohaplonts as seen in some dioecious flowering plants (*Phoenix dactylifera*, *Populus pyramidalis*). It would seem then that originally the concept and term sex expressed the idea of differentiation of somas, which were designated as the man and the woman, the male and the female. From this original meaning the terms male and female were extended, as adjectives, to the products of these differentiated somas, so that for the biologist, the products, after demonstration that cells are the effective agents in these products, became sex cells, which were called male germ cells (sperms) and female germ cells (eggs) respectively. In the case of plants, the terms male and female were later applied to the gametophyte (pollen grain, CAMERARIUS 1694). Since fusion is the apparent function of the "sex cells," and the nucleus came to be considered the essential element in the sex cell, caryogamy and reduction finally have come to be considered the sex act, and the elementary criteria of sexual reproduction and even of sex. It is apparent that in this process of extension, the criterion of the concept sex changed from differentiation of individuals to fusion of cells. From this derived point another extension was made in a different direction, in the contention that whenever cells (nuclei) fuse to form a zygote, be they differentiated or not, since this is sexual reproduction, they are sex cells, and that therefore their producers, even though they or their organs are not differentiated, are sexual individuals, either of different sex or of mixed sex. Now if we grant that because fusion occurs in sexual reproduction, fusion is the essential characteristic of sex, and that all individuals that fuse are sexual, then logically there is every reason for referring to the phenomena described for *Ectocarpus siliculosus* as relative sexuality, and to those described for Hymenomycetes as pluripolar sexuality, and for speaking of plurisexual individuals and of four, twenty, or more sexes.

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[7] ALLEN (footnote 2) points out that three categories of characters have been designated as sexual: (1) those which "arose in adaptation to the needs, imposed by the occurrence of syngamy, for securing the contact of gametes and for ensuring the sustenance and protection of the zygote"; (2) those which favor, hinder, or regulate the occurrence of syngamy; and (3) those of a chemical nature reported to exist between male and female individuals. He proposes to limit the concept and term sexual to characters of the first category. We are in essential agreement on this score.

Unquestionably there is apparent merit in the contention of HARTMANN and KNIEP that caryogamy and reduction are the essential criteria of what is called sexual reproduction or fertilization, provided one grants the validity of the extension of terms already discussed. On this basis isogamy, even of isomorphic gametes as in the Hymenomycetes, and the extreme autogamy in such forms as *Hypochnus terrestris*, are examples of sexual reproduction (KNIEP). MORGAN (59), however, points out that it is exceedingly confusing to apply the concept sex "where the phenomenon is one of union or non-union of gametes" (as in *Ectocarpus* and *Dasycladus*) "rather than one of sex as commonly understood," and suggests that the concept and term "sex factors" apply to factors "which conventionally at least apply to somatic differences in dioecious types or to those with separate sexes." PRELL (63), in discussing the phenomenon of pluripolar sexuality in fungi, is inclined to the view that it is best to avoid the term sex, because the occurrence of more than two sexes runs counter to common experience and sense. BLAKESLEE (6, 7) sensed the difficulty, and while he considered the phenomena in Mucors to be sexual reproduction, he referred to the copulants as − and +, a useful substitute for the terms male and female. DICKINSON (30, 31) tried to avoid the difficulty of referring to three sexes in *Ustilago levis* by using the term gender instead of sex.

There is a way out of this dilemma of ideas and terms. If we define sex as the *differentiation* of organisms into male and female individuals, then sexual reproduction clearly is not just any reproduction involving fusion, but only that which follows fusion of sexually differentiated individuals. Restriction of the use of the term to this sense would avoid, for example, the necessity of referring to twenty sexes in a *Coprinus*. Even when one uses the term sexual reproduction in this restricted sense, but designates all other types of reproduction as asexual and vegetative propagation, not all difficulties have been removed. The term asexual used in this sense is so inclusive that it has little exact meaning. In fact, it seems that the customary classification of reproduction on the basis of *sex* is a mistake, since it places emphasis on a subsidiary phenomenon so far as *reproduction* is concerned. An attempt is made here to devise a classification which avoids the antithesis of sexual and asexual reproduction.[8]

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[8] Originally in this article, the antithetic terms "caryogamic reproduction" and "acaryogamic reproduction" were used in the attempt to clarify the concepts sexual and

All reproductive processes can probably be grouped as amitotic and mitotic. In the Thallophytes, according to our present knowledge, amitotic reproduction occurs quite regularly in the Schizomycetes and in the Cyanophyceae, and less abundantly in some of the true fungi, such as the Saccharomycetaceae. It is premature to attempt a classification of amitotic reproductive processes. Mitotic reproduction, on the other hand, can with a fair degree of accuracy be divided into two classes: (A) reproduction which involves nuclear change, caryallagic reproduction; and (B) reproduction which does not involve nuclear change, acaryallagic reproduction. By nuclear change is meant change in nuclear relations of the cell, as formation of a dicaryon, as well as change in the nuclei themselves, such as shift from the 1n to the 2n condition. Since caryallagic reproduction results in populations of non-identical individuals, it can also be designated as "aclonogenic reproduction," and the latter, since it results in populations that are clones, as "clonogenic." The first class comprises all cases of so-called sexual reproduction and some cases of so-called asexual reproduction, while the second class comprises most cases of so-called asexual reproduction.

The following classification of mitotic reproduction is based on the course and type of nuclear behavior involved in the production of individuals.

A. CARYALLAGIC REPRODUCTION.—Reproduction with nuclear change; this does not lead to clone formation (aclonogenic).

1. Nuclear fusion is immediately followed by reduction

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asexual reproduction as applied in the Thallophytes, and to clarify sex terminology in the fungi, which appears in its most confusing form in such phrases as "quadri-sexual" and "twenty sexes." Upon consultation with Professor SEWALL WRIGHT of the Department of Zoology, after he had read this paper in manuscript, it developed that he had been concerned about the confusion existing in genetics with reference to the terms sexual and asexual reproduction. A joint attempt was then made to devise a terminology which would be acceptable to the geneticist. We started with his suggestion of using production of genetically similar or dissimilar individuals as a basis for classifying reproductive phenomena. The term acaryogamic reproduction was dropped because it covered too many diverse situations. The terms clonogenic (clone-forming) and aclonogenic (not clone-forming) reproduction were evolved by us, and a search was made for terms to distinguish the various types of reproduction under those concepts. Later the writer proposed the terms "caryallagic" (karyon, nucleus, +allage, change) and "acaryallagic" reproduction, and those subsumed under these.

(meiosis): *caryogamomiotic*[9] *reproduction*. This type of reproduction is illustrated by plants in whose life history reduction occurs in the zygote, leading to the development of a haploid body (most Chlorophyceae and fungi).

2. Nuclear reduction (meiosis) after no, one, or four equational divisions, is followed by nuclear fusion: *caryomiogamic reproduction*. This type of reproduction is illustrated by plants in whose life history reduction occurs in gametogenesis and fertilization leads to a diploid body (pennate Diatomaceae with one equational division between disjunction and fusion; Fucales with two equational divisions between disjunction and fusion in oogenesis, and five equational divisions between disjunction and fusion in spermatogenesis).

3. Nuclear reduction (meiosis) is not immediately preceded or followed by nuclear fusion: *caryomiotic reproduction*. This type of reproduction is illustrated by the so-called "asexual" reproduction in the life histories of plants with sporic or intermediate reduction. In these, according to the current terminology, the "asexual" sporophyte by reduction produces "asexual" spores which give rise to "sexual" plants, the gametophytes (*Laminaria*, *Dictyota* Bryophytes, Pteridophytes, and Spermatophytes). It is also illustrated by haploid parthenogenesis and ephebogenesis.

4. Nuclear fusion is not immediately preceded or followed by nuclear reduction (meiosis): *caryogamic reproduction*. This type of reproduction is illustrated by production of zygotes in the life history of plants with sporic or intermediate reduction. Here, according to the current terminology, the "sexual" gametophyte through the process of fertilization produces zygotes which give rise to "asexual" sporophytes. The examples are the same as those listed under caryomiotic reproduction, excepting haploid parthenogenesis.

5. Nuclear pairing which is not immediately followed by fusion precedes reproduction: *caryozeugotic reproduction*. This type is

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[9] This and the following terms have been synthesized from karyon = nucleus, gamo = fusion, and meiosis = reduction; anomos = irregular, mitosis = equational division; cytos = cell, blastos = bud, genes = producing; zeugos = yoking, pair; lysis = loosening, separation. Nouns can be made from these terms by addition of the suffix -gony.

illustrated by some fungi in whose life histories cell fusion or nuclear differentiation (as in *Hypochnus terrestris*) leads to a dicaryophase. This does not involve change so far as the nuclei are concerned, but it does involve change in nuclear relations of the cell which are significant morphologically, physiologically, and genetically. Thus such dicaryophytes are often more virulent than the haploid phase, and show dominance phenomena where genotypically different nuclei are involved. Clear-cut caryozeugotic reproduction is illustrated by gametogamy in *Taphrina epiphylla*, *Tilletia tritici*, and other Ustilaginales; by gametangiogamy in various Phycomycetes; by somatogamy in aeciospore formation in *Phragmidium speciosum*, by uredospore formation in *P. potentillae canadensis*, and all other cases in rusts in which nuclear pairing directly leads to spore formation.[10]

6. Nuclear separation occurs in the dicaryophase before fusion occurs: *caryozeugolytic reproduction*. This type occurs in fungi in whose life history the dicaryophyte produces uninucleate (1n) individuals by separation of the paired nuclei. It is illustrated by the formation of basidiospores in *Kunkelia nitens*, and by formation of uninucleate oidia in *Ustilago levis*.

7. Nuclear irregularities occur during mitosis: *anomomitotic reproduction*. This includes a great diversity of phenomena during cell division which may play a rôle in mutation. Illustrations are unequal distribution of chromosomes, both as to number and kind; failure of chromosomes to separate after division; unequal changes in homologous chromosomes and genes.

B. ACARYALLAGIC REPRODUCTION.—Reproduction without nuclear change; this leads to clone formation (clonogenic).

1. Reproduction by cell division: *cytogenic reproduction*. This is illustrated by equational or ordinary cell division, production

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[10] It is debatable whether every case of pairing of nuclei (caryozeuxis) which is not followed by immediate fusion and which leads to a binucleate mycelium should be classed here. In so far as a new generation is produced, it would be correct to do so. However, generally the concept reproduction implies the formation of a new and distinct individual. If one chooses to designate all these cases as caryozeugotic reproduction, then the term should be reserved for the first cell that is formed by division of the dicaryon, because further cell division and spore formation by this dicaryon and its descendants do not involve nuclear change, and are a type of acaryallagic reproduction.

of agametes, that is, zoospores, sporangiospores, conidiospores, etc., also diploid parthenogenesis and ephebogenesis.

2. Reproduction by budding and vegetative propagation: *blastogenic*[11] *reproduction.* Sclerotium production is an illustration among the fungi. It is debatable whether the acaryallagically produced multicellular spores of fungi should be considered cytogenic or blastogenic reproduction.

It is apparent that this system enables one to classify reproduction without resort to the concepts sexual and asexual. Sexual reproduction would occur only as a *special* case in caryogamomiotic, caryomiogamic, caryogamic, and caryozeugotic reproduction when sexual differentiation of the copulants or of their producers is involved. In other words, this classification enables one to stress differentiation and not fusion as the characteristic feature in sex. It also indicates that the usefulness of sex must be sought in something other than mere reproduction. Abandoning the terms sexual and asexual reproduction would avoid, for example, such inconsistencies as the contention that a sexually differentiated sporophyte (*Phoenix dactylifera* for instance) or the sexually differentiated organs of a sporophyte (floral organs of any flowering plant) *asexually* produce sexual generations, the gametophytes, which *sexually* produce the sporophytes, which are, however, referred to as asexual. The definition of alternation of generations also can be restated as any periodic or more or less irregular alternation between generations with any two kinds of reproduction. This removes the obligatory association of alternation of generations and alternation of nuclear phase.

The general term copulant proposed by KNIEP (55) can be retained, because in itself it does not connote sex. If one chooses to define a gamete as does KNIEP, caryogamic, caryomiogamic, caryogamomiotic, and caryozeugotic reproduction can be considered to involve reproduction following gametogamy, gametangiogamy, or somatogamy, which may be isogamous or anisogamous. If one chooses to designate as a gamete any cell whose nucleus fuses, or any nucleus which fuses with the nucleus of another cell or with another nucleus, then these types of reproduction, excepting caryozeugotic, can be considered coextensive with "reproduction following gametic union," a phrase used extensively by KRAUS (57), or as "gametic reproduction." It seems preferable, however, to differentiate between gametes, gametangia, and soma as copulants, and to indicate the time relationship between copulation and meiosis, certainly in fungi, and consequently the phrase "reproduction by gametic union" is not a substitute for caryogamic, caryogamomiotic, or caryomiogamic reproduction.

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[11] Unfortunately the root "blastos" which means sprout, sucker, shoot, has in zoological literature been used in a derived sense, that is, as germ. As a general biological term, somatogenic might therefore be preferable.

Copulation and sterility factors can be hypothecated for homothallic (monoecious) forms with isomorphic copulants without introducing the concepts sex, sexuality, or sexual reproduction. When the level of unequal or unlike copulants is reached, one can speak of micro- and megacopulants (micro- and megagametes in anisogamety), using form, size, metabolism, or behavior as criteria, without any sex connotations if one wishes. On the other hand, one may interpret this as the level at which the simplest type of sex emerges, because there is differentiation of copulating cells or organs. At the next level emerges differentiation of the gamete containers as micro- and megagametangia. This is followed by differentiation of the entire soma which bears the copulants, as for example, when true gametes are borne, of the gametophyte into micro- or megagametophytes. At this stage one can truly speak of sex, in the sense that there is differentiation as male and female of the individuals which bear the copulants. Finally, the differentiation may even extend to the diploid soma which produces the copulant or the copulant-bearing soma (gametophyte).

It appears then that sex is a special adaptation which becomes associated with reproduction relatively early in the phylogenetic series if one considers differentiation of copulants as the criterion of sex; and relatively late, if one considers differentiation of the copulant-bearing somas as the criterion (MORGAN 59).

Rationalization of caryallagic and acaryallagic reproduction

Can sexuality be explained causally, as HARTMANN attempted? What is the significance of its association with reproduction? Caryallagic (aclonogenic) reproduction, especially as represented by caryogamic and caryomiotic reproduction in combination, and by gamocaryomiotic, caryomiogamic, and caryozeugotic reproduction, is a means whereby organisms acquired the mixing of germ plasms, amphimixis, and exploited its advantages. Consequently it may be considered a special adaptation. Now amphimixis not only tends to level differences between individuals, but it also provides means for enhancement of individual differences as a result of new combinations of factors. In this way it seems on the one hand to provide stability, and on the other hand, plasticity and diversity within the species. In association with mutation it becomes a powerful factor for plasticity and diversity, and with isolation, of species formation. It would seem therefore, that a species with reproduction involving nuclear change would tend to show diversity, and that in a changing environment this would be of survival value.

Caryallagic reproduction seems to have arisen independently again and again in the Thallophytes. Its manifestation, in spite of great diversity, falls into fairly general patterns which appear in the various series. One pattern seems to be the attainment of intensive amphimixis; another, the prevention of intensive amphimixis. The former is attained by cytogamy and caryogamy ranging from union of the copulants that are genetically alike to union of those that are genetically different; the latter by the reverse series. One can construct, as PRELL (63) has done, a series ranging from fusion of sister cells derived from a haploid mother cell, haploid autogamy (automixis), through fusion of cells derived from the same zygote, endogamy (endomixis), to fusion of cells derived from different diploid soma, exogamy (exomixis). Various devices and processes that occur again and again are employed not only to make endomixis and exomixis possible, but even to render them obligatory. These devices may be phenotypic, as in anisogamety, heterothallism, or heterophytism (heterozygotism). In anisogamety there may be extreme differentiation of the microgamete for mobility and of the megagamete for food storage at the extreme end of the series. These devices may also be genetic in the form of Mendelian copulation or sterility factors and heterogamety. Considered in this fashion, caryallagic reproduction makes amphimixis possible and various special adaptations, such as sex, which have become associated with it, foster amphimixis and resultant diversity, and possibly enhanced chances of survival.

EAST (34) has pointed out that if n number of variations occur in an organism reproducing caryallagically, then 2^n number of types can be formed as a consequence of fusion and reduction, whereas in an acaryallagically reproducing organism, only n number of types can be formed. Thus if ten variations occur in the latter, only ten types can possibly result, whereas the same number of variations in the former have a maximum possibility of 1024 types. This is the situation when caryogamomiotic reproduction occurs, the zygote being reduced immediately after its formation, as in many algae (Conjugatae, Chlorophyceae, and haplobiontic Florideae), and in some fungi (Phycomycetes).

Caryallagic reproduction has exploited the advantages of amphimixis even more extensively in those organisms in which there is an extensive development of a diploid soma. In some of these forms, fusion of nuclei is not immediately followed by reduction, or cytogamy is not immediately followed by caryogamy or by dissolution of the dicaryon. The advantage of this lies in the fact that, whereas 2^n number of types may result when immediate reduction occurs, 3^n number of types may arise if a diploid body results and mitotic divisions of the 2n nucleus or conjugate divisions of the dicaryon are inserted between the fusion of the nucleus and reduction, or between cytogamy and caryogamy and reduction, so that great numbers of gonotoconts (spore mother cells, asci, and basidia) may result from one fusion, each reduction in turn carrying potentialities of new combinations.

To put it in another way, such organisms have a better chance of exploiting all the possible types of variations than do those in which reduction immediately follows nuclear fusion. SVEDELIUS (72) has pointed out that a plant with n number of chromosomes in the haploid state, and consequently 2n number in the diploid state, will have 2^{n-1} number of possible different combinations of haploid nuclei, and that 2^n is the theoretically minimum number of reduction divisions necessary to realize all the possible combinations. Thus "a plant with 10 chromosomes in the haploid nucleus, and consequently 20 in the diploid, can by reduction divisions so combine them that 1024 ($=2^{10}$) different haploid nuclei can be formed, and for this at least 512($=2^{10-1}$) different reduction divisions are required." Delay of reduction (caryomiotic in combination with caryogamic, and caryomiogamic reproduction) occurs in the algae, which are characterized by the greater abundance of forms and species and higher differentiation of the thallus (diplo-biontic Florideae and Phacophyceae, also pennate Diatoms). Delay of fusion of nuclei (caryozeugotic in combination with acaryallagic and caryogamomiotic reproduction) is most marked in those fungi which have the greater number of forms and species, and the highest differentiation of the thallus. In the Phycomycetes the dicaryophase is relatively brief. In the Euascomycetes, development of the fruiting body is either initiated by the processes of nuclear fusion, or it is preparatory thereto, the soma however being haploid. In the Basidiomycetes the fruiting bodies are part of the dicaryophase, and are formed preparatory to caryogamy and reduction. Even in forms with extreme autogamy, as in *Hypochnus terrestris* and some Gasteromycetes, it is conceivable that the increased number of nuclear fusions and reductions may affect combinations of the factors resident in the one nucleus whose division leads to conjugate nuclei.

We have seen that caryallagic reproduction is an arrangement whereby reproduction and amphimixis are associated, and is a means of obtaining diversity and plasticity which may be of survival value in a changing environment.

On the other hand, acaryallagic reproduction can be considered a means whereby reproduction and apomixis (in the broad sense of PRELL and the restricted sense of KNIEP) are associated, and the advantages of the latter exploited. This tends to reduce diversity and to perpetuate the strain unchanged. It would seem that in an unchanging or relatively stable environment this situation would be of value to organisms that find themselves adapted, for they do not run the chance of losing a favorable variation by reshuffling of factors as soon as this is realized, as they do in amphimixis. Elimination of the possibility of new combinations would also be of advantage, because most variations and new combinations seem to be useless if not harmful. Consequently one would expect a variety of arrangements which make apomixis possible and obligatory, or which tend to decrease the intensity of amphimixis, or even to eliminate it, in organisms which possess the facilities for caryallagic reproduction. One of these is persistence of acaryallagic reproduction as in fungi. Another is the quantitative decrease of caryallagic reproduction and the corresponding or even greater increase of acaryallagic reproduction as in fungi. Another is a series wherein the intensity of amphimixis is progressively diminished as in fungi (Ascomycetes) with parthenogamy, and those with extreme autogamy in the soma, as in *Hypochnus terrestris*. Another is apomixis in the restricted sense of KNIEP, in the form of gametic, gametangial, and somatogenous apomixis, all of which are represented in the fungi to a marked degree.

Finally, the chances of survival of a species or race in an environment which is essentially fixed but which either changes slightly now and then or, as is the case in all environments, is likely suddenly to change appreciably, would seem to be best if it combines the advantages of acaryallagic reproduction with those of occasional caryallagic reproduction, or at least keeps the latter in reserve. This situation we find in many successful parasites and pathogens, such as fungi and insects, for example. This arrangement may be of significance in those fungi which are characterized by an abundance of physiological species, varieties, or races, and in which both caryallagic and acaryallagic reproduction occur; the former as an occasional process, thus serving to produce new genotypes in heterothallic forms, and the latter to propagate, spread, and conserve the genotypes which find themselves adapted to their hosts. This may be the situation in some of the Erysiphaceae and Uredinales. In case CRAIGIE'S reports (**26, 27, 28**) of heterothallism for *Puccinia graminis* are substantiated, this fungus may be a case in point when its complete life history is realized, with its intense specialization of races, its great display of acaryallagic reproduction on diverse hosts, and the comparatively infrequent inauguration of caryallagic reproduction by the various strains on the common alternate host. On this basis the greatest display of genotypes of *Puccinia graminis* ought to exist in those regions where an abundance of barberry and a great diversity of grasses are combined.

We thus achieve a rationalization of caryallagic reproduction and sexuality in Thallophytes, in that they appear to be factors which foster survival and evolution. Sex is only one, although an important means of exploiting caryallagic reproduction. Caryallagic reproduction, especially in association with sex, seems to play a rôle in producing adapted organisms by favoring plasticity, diversity, and differentiation. This is not a causal explanation, since rather than telling us what really conditions caryallagic reproduction and sex, it points out what they accomplish, and savors of teleology in that it imputes purpose.

But even from the ecological point of view the situation is not unequivocal, for together with organisms which reproduce caryallagically are others, apparently equally successful, which do not reproduce in this manner. If we measure success on the basis of ability to survive in diverse and trying habitats, certainly the bacteria and the lichens, which are either without caryallagic reproduction or without intense amphimixis, are as successful as are Thallophytes which have intense amphimixis.

In caryallagic reproduction and sex, we find two opposed and apparently contradictory tendencies at work. One leads to intense amphimixis, ranging from automixis to exomixis; and the other to slight or no amphimixis, ranging from exomixis to automixis, or even to elimination of caryallagic reproduction, as in apomixis.

The pursuit of a single definite conclusion with regard to the causality of caryallagic reproduction and of sex will continue so long as curiosity concerning them exists, and will lead to ideas and experimentation with the promise of results of theoretical and practical significance. The pursuit may well prove endless, and we may have to say with WILLIAM JAMES, "There is no conclusion. What has concluded that we might conclude in regard to it?"

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[1] For a very full bibliography, see Kniep, 55 (about 2000 citations).

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DEVELOPMENT OF ANTHERIDIUM AND SPERMATOZOID IN PLAGIOCHILA ADIANTOIDES LINDB. (SWARTZ)[1]

DUNCAN S. JOHNSON

(WITH PLATES I–III AND FOUR FIGURES)

Introduction

This study of the development of the antheridia and spermatozoids of *Plagiochila adiantoides* was made upon plants which were found growing on decidedly moist soil in the damp passes or on the forested peaks of the Blue Mountains of Jamaica. It was from this island that this species was originally described by SWARTZ (LINDENBERG and GOTTSCHE 8). It occurs at altitudes ranging from 4500 to over 7000 feet. Shaded soil here may often be almost or even completely covered with this liverwort over areas of several square yards. The stems grow to 10 or 12 cm. in height, are only sparsely branched, and may stand erect or half-erect, or lie nearly horizontal. The plants of any one clump seem to spread by continually growing forward about the edges of the patch while dying off at the center or basal end. Wherever branching occurs, the death of the parent branch which follows two or three years later leaves the two branches as free, independent plants. This type of vegetative propagation, common among creeping bryophytes, evidently results after decades or perhaps centuries in suitable habits, in the formation of patches of hundreds or thousands of plants all descended from the one or the few pioneers that first established themselves at the spot. Clumps half a meter across may thus often be formed, each of which consists, in so far as the plants are fertile, entirely of antheridial plants or entirely of archegoniate ones.

Sexual reproductive organs and spore capsules were not found on more than 10 per cent of the plants In most patches, at least between April and July when the plants for this study were collected, the percentage of antheridial and archegonial plants together

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[1] Botanical contribution from the Johns Hopkins University, no. 90. The collection and preparation of material for this study were done by the aid of a grant from the Bache Fund of the National Academy of Sciences. Much of the work on the slides and manuscript was done at the Mt. Desert Island Biological Laboratory.

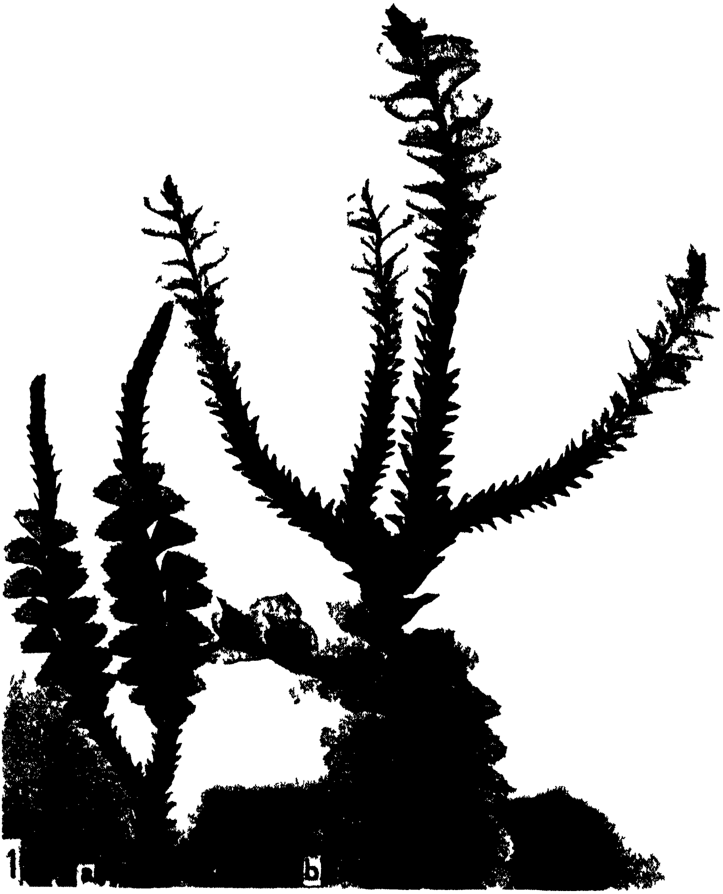


FIG 1 —*a* photograph of tip of male plant from above showing forked series of involucral leaves below and another distinct series on tip of each branch, at base and along middle of each branch are sterile photosynthetic leaves, ×2 6, *b* view from above of twice forked tip of antheridial plant showing structure of normal photosynthetic leaves below, and others above lower antheridial spikes, reduced to half normal size This alternation of groups of normal and fertile leaves on same axis is of frequent occurrence Antheridia have been discharged from all involucral leaves except those at very tips of spikes, ×3

was decidedly less than this. The plants used for this study were collected in various habitats in the Blue Mountains of Jamaica, on Blue Mountain Peak (7000 ft.), or at Morces Gap (4943 ft.) and New Haven Gap (5400 ft.) near the Cinchona Botanical Station.

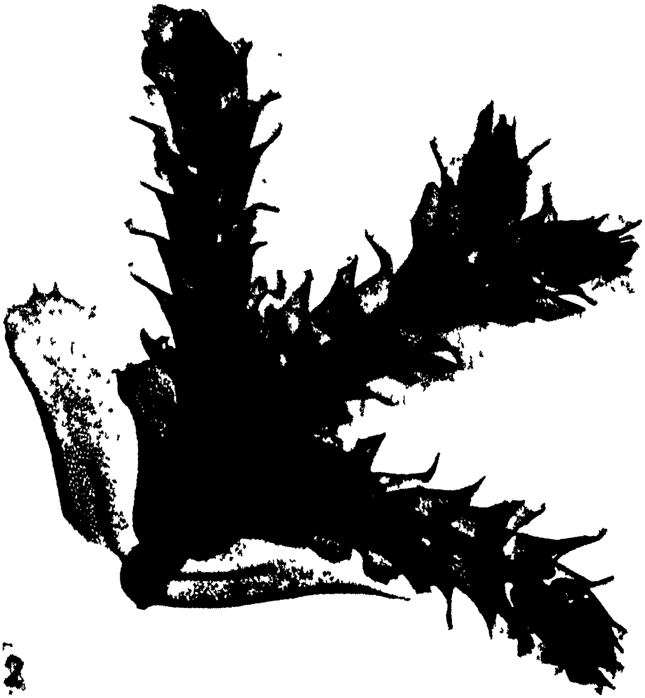


FIG. 2.—Thrice-forked antheridial spike seen from above, showing cellular structure of sterile leaf, half urn-shaped form of involucral leaf, and position and relative size of antheridia; ×10.

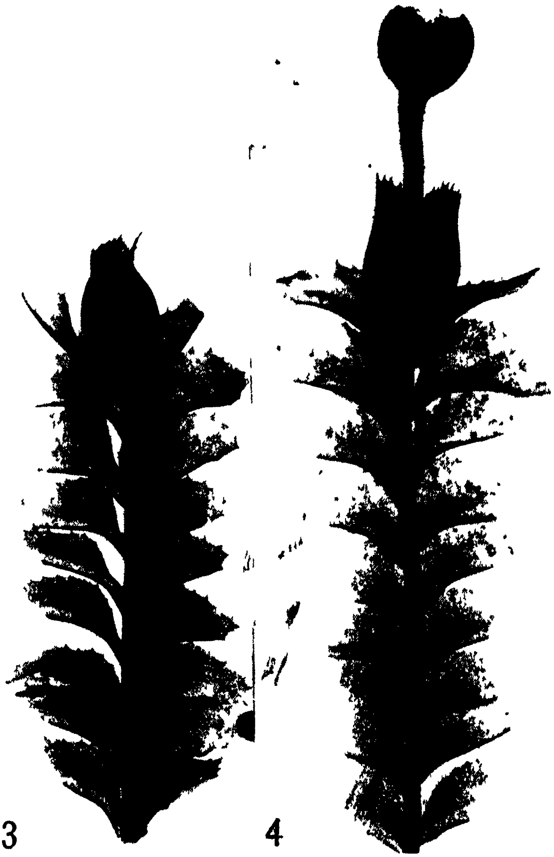
They were fixed at various hours of the day, from 7:00 A.M. to 10:00 P.M., some in a medium chromoacetic fixing fluid and some in normal Flemming's fluid, and still fewer (1926) in formol-alcohol-acetic. In some cases fixation was done in the field. More commonly the plants were carried back to the laboratory in a tin box and then either fixed the same day or kept overnight in large, covered glass dishes, to be fixed early the following morning. After the return to Baltimore the material was imbedded in paraffin, sectioned and stained in Flemming's triple or with Haidenhain's iron haematoxylin.

I. VEGETATIVE STRUCTURE

The vegetative plants of *Plagiochila adiantoides* have rather straight, rigid, often reddish stems which branch only infrequently in the free, exposed parts. Deeper down in the mat or turf of radiating, overlapping shoots, the forkings which give rise to the many free aerial shoots can be found. These terminal aerial branches may grow to a decimeter or so in length without branching. They are strikingly flattened and dorsiventral, and each bears from 40 to 100 markedly toothed, succubous leaves, which range in length from 3 to 5.5 mm. and in width from 2 to 4 mm. (figs. 1, 4). Rhizoids are never abundant. Those found are located at the bases of the frayed-out leaves, at least 10 or 12 cm. back from the apex of the branch bearing them. No rhizoids at all were seen on exposed parts of the stem which bore still functional leaves. It seems clear, from the sparsity and the small size of the rhizoids here, that this liverwort must take up most of the water and salts needed through the leaves themselves. The relatively large leaves of this species are usually pale green or yellowish in color. They are rarely as deep green as *Scapania nemorosa* becomes when moist.

II. ANTHERIDIAL PLANT

The antheridial plant, or at least the fertile, aerial branches of it may, even in its still vegetative portion, be rather more slender than the sterile plant, that is, have shorter and often narrower leaves, although male plants are not always smaller plants. The fertile antheridium-bearing portion of the male plant may be simple, and 10–30 mm. long (fig. 1*a*). In other vigorous plants the fertile apex of the male plant may fork once, twice, or even three times in rapid succession, and thus, with further equal growth of the several branches, may give rise to a fanlike terminal group of antheridial spikes (fig. 1). In still other cases one or more of the primary divisions of the spike may itself divide in the midst of the series of involucral leaves to two, thus giving rise to secondary spikelets (figs. 1, 2). The branching in all such cases, as is evident at the beginning, is truly monopodial. Later in the development, however, it may come to appear dichotomous.



Figs. 3, 4.—Fig 3, tip of archegoniate plant from above, showing normal leaves below and involucre at tip with inclosed sporogonium, ×6. Fig. 4, tip of archegoniate plant, seen from above, showing opened involucre, fully elongated seta, and ruptured capsule with protruding elaters and spores; ×6.

Not infrequently the axis of a male spike, after developing a score or two of involucral leaves, may begin to form a series of often a dozen or a score of foliage leaves. These may then be followed by a second series of antheridium-bearing leaves on the same continuous branch, and these in turn by another series of foliage leaves. Whether both the fertile and sterile sections of the stem may be developed during the same growing season was not definitely determined. The foliage leaves developed above an antheridial spike are commonly not as large and vigorous as those of the basal part of the same shoot. In smaller plants they may be nearly so.

The antheridial spike may be of any length, from 10 to 25 or even 35 mm. In width it ranges from 1 to 2.5 mm. The transition from a vegetative branch to a male spike or the reverse may be quite abrupt, so that the limits of the two are clearly distinguished (fig. 1*a*). In other rarer cases the leaves of the vegetative portion of the shoot gradually become smaller through a series of two or three, or sometimes of twenty or more, leaves as we follow up the stem, until the antheridial branch has been reduced to only one-fourth or one-fifth the width of the foliage shoots, and the bases of the leaves have become deeply concave toward the stem (figs. 1*b*, 2). Antheridia are found only on such highly modified leaves, usually one in each axil. The transition from the antheridium-bearing to the normal foliage leaves, often found above them, may in like manner be either abrupt or more gradual (figs. 1*a*, *b*). The alternation of antheridium-bearing and sterile sections of the stem of the male gametophyte resembles strikingly the alternating sporophyll-bearing and sterile sections of the stem of the sporophyte in such club mosses as *Lycopodium lucidulum*.

The number of these involucral or antheridium-bearing leaves that may be formed in one continuous series on a single spike ranges from ten or twelve to thirty or forty, or more rarely even up to fifty or sixty leaves on each side. These leaves are borne in two ranks, one at the right and one at the left of the stem; that is, a spike of forty involucral leaves would have twenty of these on each side. These leaves are, of course, alternate in origin at the growing point. Sometimes they retain this distribution when mature (figs. 1*a*, 2, 36); in other cases, however, successive involucral leaves of the right and left sides of the spike are almost opposite, thus forming apparent pairs. The leaves of such a pair may be separated by very short internodes, only one-fifth as long as the stretch of axis separating this pair from the next one above or below (fig. 2).

The antheridium-bearing leaf is not nearly flat, as the foliage leaf is except for its concave base, but the involucral leaf is curled upward from the dorsal (or upper side) of the stem, and then outward, next downward, then inward to the stem; and finally it turns downward again at the tip to form a protective structure with a half-urn-shaped base and a widely flaring margin. This margin is serrate, like that of the foliage leaf (fig. 2). The ventral edge of the involucral leaf extends vertically downward, so that these edges of the successive involucral leaves form a composite keel, four or six leaves thick, projecting from the lower side of the strongly dorsiventral spike (figs. 13, 16).

One antheridium only, with rare exceptions, is borne in the axil of each involucral leaf. In a very few cases two antheridia were present on one involucral leaf, where they had evidently developed successively. The younger antheridium in all such cases has a stalk that is inserted above, that is, nearer the stem apex than that of the older antheridium (figs. 9, 20, 36, 47). In none of the hundreds of cases studied were more than two antheridia present in the axil of a single leaf. It would be interesting to know the developmental sequence of the six or more antheridia shown by GOEBEL (5) to occur above each leaf in the antheridial spike of the related genus *Tylimanthus*.

The body of the mature antheridium is globular or slightly ellipsoidal in form (figs. 32, 34, 36). The length of its stalk is, for a long time, about equal to the diameter of the body of the antheridium. The stalk stands off at a sharp angle (45°) with the stem, and the body of the younger antheridium may often lie in contact, both with the inner surface of its own involucral leaf and with the outer surface of the next higher leaf (figs. 13, 36). A single cross-section of the younger part of an antheridial spike may pass through as many as four involucral leaves and two antheridia, or the stalks of these, on each side of the stem. The older antheridia often lie well above the level of the dorsal surface of the stem of the spike.

The persistent growth of the antheridial spike means that antheridia of many different ages may be found along its length. Thus in figs. 36 and 50 the basal antheridia are practically mature, while in the apical ones there visible the body and stalk are just being differentiated. Still other spikes may bear only remnants of ripened and discharged antheridia below and very young few-celled rudiments above. The largest number of antheridia seen along a single, simple spike is twenty-two. These counts were made in preparations cleared in balsam or glycerin, and included all antheridia present from the smallest visible rudiment up to the most mature, still undischarged antheridium on both sides of the axis. In microtome sections where smaller rudiments can be seen, the total number discoverable in one spike may be 24–26. The number of antheridia that may be developed in a single growing season was not determined. In some habitats the growth of the plant may well be practically continuous, and antheridia may thus develop in regular succession all through the year.

III. FEMALE PLANT

The archegoniate or female plant of *Plagiochila adiantoides* is commonly unbranched for a distance of 2–8 cm. back from the apex. The archegonial plants are frequently, although not constantly, distinguishable from the male by their greater robustness. The leaves of the female plant are unmodified except for the last two (one on each side) at the very tip. These two leaves are not expanded to right and left of the stem as those of the antheridial spike are, but remain pointing forward as all the leaves do when they arise in the bud. They are nearly vertical, rather than horizontal like the foliage leaves, but they are also strongly concave inward, which is likewise the form they came to have while still in the bud (figs. 3, 4). Although usually somewhat compressed from side to side, the involucral leaves do not fuse at the margins to form a continuous, cornucopia-like perianth as in many other foliose Jungermanniaceae.

The archegonial branch itself is always simple, so far as observed; never forked as the antheridial branches commonly are. Since usually but a single archegonium in each involucre is fertilized, there is but one sporogonium developed on each female branch. Two have been found in one involucre in not more than half a dozen cases out of hundreds seen. Since the archegonial involucre points straight out apically, a plane passing longitudinally through the axis, parallel to the leaves, would run through the group of archegonia.

IV. DEVELOPMENT OF ANTHERIDIUM

The antheridium initial, or mother cell, is often recognizable in the next to the youngest segment of the apical cell of the stem. The antheridium mother cell evidently arises from the acroscopic half of the stem segment, while the subtending leaf comes from the basiscopic half (fig. 8). The two walls first formed in the young antheridial rudiment are transverse. Of these, the first cuts off a basal cell (fig. 9), while the second wall, in the upper cell, separates a lower or stalk cell from a terminal or body cell (fig. 8). The first longitudinal wall in both the stalk and the body cell is a median or diametric one, which is usually approximately tangential to the upper right or upper left flank of the stem (figs. 8, 10, 13, 16). This first median wall usually makes an angle of about 45° with a horizontal plane, that is, with a plane lying parallel to the stem and through the antheridia of its opposite sides (figs. 13, 16, 20, 38, 43, 49). In fewer cases this wall may be more nearly vertical (figs. 39, 40) or horizontal (figs. 42, 48). In the stalk there is commonly no other longitudinal wall formed. Even the mature antheridium has a stalk which is still but two cells wide (figs. 17, 46).

The second longitudinal wall formed in each half of the young antheridium, the "quadrant wall," is often approximately radial, or in others more nearly periclinal. At its basal edge this is nearly perpendicular to the transverse or basal wall, which separates the antheridium from its stalk (fig. 24). This second wall cuts in two each hemispherical half of the antheridium (figs. 39, 43). This quadrant wall is often sharply concave, as is shown by the curved line (a quarter circle) in which it intersects the plane, median or diametric wall. It intersects the outer cell wall of the hemisphere in a less sharply curved line (figs. 8, 10, 27). The quadrant walls of the two halves commonly make approximately a right angle with each other (figs. 40, 41, 42). The second wall to be formed in each half of the antheridium is oftenest a very nearly periclinal one arising in the larger quarter (figs. 40, 41). The outer cell thus formed completes the protective antheridial wall of four cells in its circumference, and leaves a single, rather angular, half-dome-shaped primary spermatogenous cell in each half of the antheridium (figs. 20, 24, 41).

The further development of the wall of the antheridium results in the formation, by repeated anticlinal divisions, of a large number of protective cells in a single layer (figs. 23, 27, 29, 33*a*, 34, 48). These cells remain practically isodimensional until the antheridium is nearly mature (figs. 32, 35, 49). In the latest stages of development, while the radial and transverse dimensions of the cells of the wall remain about equal and constant, they often become appreciably elongated in the meridional direction (figs. 33*a*, 34). The total number of wall cells seen in cross and in longitudinal section of the mature antheridium is nearly the same, about 25–30. The inner and outer walls of these cells are only slightly different in thickness. Nevertheless they show a striking change in form at maturity, with the increasing turgor of the wall cells, similar to that which is known in antheridia of *Pallavicinia* and other liverworts (GOEBEL 4). The result of the greater stretching of the inner walls of these cells by turgor is the complete eversion of the several irregular flaps into which the upper half of the wall of the antheridium is torn when it bursts, until these flaps are bent outward and often downward toward the stalk of the antheridium. This is shown in fig. 35, which was drawn from a living antheridium in Jamaica in 1926.

The length of the capsule of the mature antheridium ranges from 0.2 to 0.4 mm. The length of the stalk is from one-half to three-fourths that of the body of the antheridium. The stalk shows no marked stretching at maturity, and the spermatozoids are therefore discharged within the cavity formed by the hollow involucral leaf. They must evidently make their way out (to reach archegonia) through the rather narrow crescentric slit between their own involucral leaf and the next leaf above this (figs. 13, 36). The mature spermatozoid within the androcyte finally becomes coiled to somewhat less than two complete turns (fig. 98). The maximum diameter of the body of the spermatozoid is about 1 μ, and its total length about 35 μ.

The later development of the single primary spermatogenous cell, formed as described in each half of the antheridium, may now be followed. The somewhat angular half-dome-shaped cell in each half of the antheridium (figs. 24, 41) is, before it has enlarged greatly, divided by an anticlinal wall. This first wall is often longitudinal to the antheridium and practically perpendicular to the median, diametric wall (figs. 20, 42, 47). Transverse and other longitudinal and also diagonal anticlines and then periclines follow rapidly as the antheridium develops (figs. 11, 25, 26, 30). Later variously oriented walls appear all through the antheridium (figs. 31, 32, 33*a*, 49). Thus the two primary spermatogenous cells of the antheridium divide successively by walls in all planes, until there are from 700 to 800 "androcyte mother cells" present in the surface of a median longitudinal section of the antheridium and about 700 in a median cross-section. Since each androcyte mother cell forms two androcytes and then two spermatozoids, this of course means a total of some 25,000, to 30,000 spermatozoids from each antheridium (figs. 19, 33*a*). Before the final division each of these mother cells becomes rather cubical or somewhat polyhedral in form, and nearly isodimensional. The spindle of the final division in these cells more commonly, although not constantly, lies in one diagonal plane of the cube, and the plane of the resulting cell division thus lies in another diagonal (figs. 19, 82, 86).

The cytoplasm of all spermatogenous cells, from the beginning up to the young spermatozoids themselves, is rather finely vacuolated, and contains numerous very fine granules (figs. 51, 54, 55*a-c*, 70, 80). No plastids and no granules of distinctive size and form are at all constantly present, and no larger cytoplasmic inclusions were discoverable until the possible blepharoplasts, to be mentioned later, had appeared in the last mitosis. A larger, faintly staining, dark body (a limósphere?) sometimes becomes evident in the androcyte, after this mitosis (fig. 94*a*). The most careful search in hundreds of androcyte mother cells, in all stages of development, served to discover but few cases where anything comparable to a centrosome or blepharoplast could be clearly distinguished (WOODBURN 14). Even in those cases the minute, darkly stained body could not be distinguished until after the mitotic spindle of the very last division had already been organized; and then such a more pronounced dark dot could usually be found near but one of the two poles of the spindle (figs. 80, 82). Younger androcyte mother cells show a nearly spherical nucleus, with peripheral chromatin granules of various sizes in a more or less distinct reticulum (fig. 69). Slightly later the chromatin thread thickens, its anastomoses become few, and then it breaks up to an at first inconstant number of loops and rods (figs. 71, 72, 73). The chromatin structure found in the resting nuclei of the preceding generation of spermatogenous cells (figs. 54, 63) can also be seen in very early phases of the androcyte mother cell nucleus. The nucleolus, usually present in the nucleus of earlier generations of androgones, as well as in that of cells of the wall of the antheridium (figs. 51, 54, 63) does not stain so distinctly in the nucleus of the androcyte mother cell (figs. 56, 57, 69).

Development of spermatozoid

The androcyte, as will be evident from what was just said of the form of its mother cell and of the position of the mitotic spindle in the latter, forms commonly a triangular prism. The base of each prism is one-half of one face of the cube, and the broadest face is that lying next to its sister androcytes (figs. 33*a*, 86). The cytoplasm of each ultimate androgone, "androcyte mother cell," just before its division, is regularly and finely vacuolated (figs. 63, 70). The cytoplasm of the daughter androcytes (young spermatozoids) is distinctly vacuolated at first (figs. 84, 86), but this commonly becomes somewhat less evident as development progresses and the protoplast elongates (figs. 58*a*, 86, 94).

The nucleus of the very young spermatozoid, when first organized after the final mitosis, is nearly globular (figs. 55*b*, *c*, 84, 86). The nuclear wall is distinguishable before the spindle fibers have disappeared, and remains clear until the developing spermatozoid has begun to coil (figs. 55*b*, 64, 87, 94). The chromatin of the young androcyte mother cell is found in rounded, or slightly elongated roughish grains (figs. 62, 63), which even at first are connected by but few visible strands of stainable substance (figs. 63, 70). The number of these grains, which differ markedly in size, is at least twice that of the chromosomes. Even these small grains seem composed of still more minute granules, as becomes more evident when the chromosomes for the last mitosis are organized (figs. 79, 83, 85). The nucleolus, which is evident in resting nuclei of all spermatogenous cells, up to the young androcyte mother cell (figs. 54, 62, 63, 69), disappears with the organization of the chromosomes at mitosis (figs. 55, 72, 76). No clear evidence was obtained of any exchange of material between nucleolus and chromosomes.

The changes in organization of the nucleus of the androcyte mother cell before the final mitosis include a disappearance of many of the stainable connecting strands between the numerous chromatin grains. This is followed by the aggregation of all the chromatin into a finally definite number of evidently composite granular masses, which are later to become the chromosomes (figs. 69, 70, 73, 77). The smaller, more numerous grains (fig. 69) are often angular or irregular in shape, are peripheral in position, and are definitely connected to an irregular reticulum, in which they lie at the nodes. These grains, by their decided roughness, show some signs of being composite, but they are not evidently hollow, as the larger aggregations soon to be formed often are (figs. 71, 75, 79).

The next evident step in the progress of this last mitosis is the aggregation (the further collection) of the chromatin granules to form the rodlike and often hollow chromosomes. When first formed the latter are evidently composed of a number of granules each (figs. 73, 79); somewhat later the component granules have become less distinct, and the still oblong chromosomes seem to have a nearly continuous dark outer layer, with a less deeply staining central region (figs. 76, 78). This difference in staining capacity of the axial and peripheral portions of each chromosome persists, and is clearly evident whenever the stain is not too intense (figs. 76, 79). Not only is this true, but in well stained nuclei the peripheral chromatin can still be seen to be composed of grains which often seem to be grouped in transverse bands around the circumference of the chromosome (figs. 74, 79). In more deeply stained chromosomes these chromatin granules may be indistinct, or may even be quite indiscernible (figs. 75, 77, 78). In the chromosomes as grouped for the mitosis, from prophase to anaphase, the chromosomes have become shortened to about half their original length, and seem practically solid when stained to show at all clearly (figs. 77, 80, 82).

Many mitoses in spermatogenous cells were studied in search of any constant difference in size or form among the ten chromosomes of any individual nucleus, or differences (in size or number) between the chromosome complements of different spermatozoids. Neither type of difference was discovered; that is, no differences were found in the chromosome complements of different spermatozoids that might be correlated with differences in sex, or in other qualities, in the individuals produced by eggs fertilized by these different spermatozoids (ALLEN 2, SHOWALTER 13, and MCALLISTER 9). Unfortunately no meioses were found of spore mother cell nuclei with their probably larger nuclei and chromosomes.

At the equator of the last mitotic spindle in the spermatogenous cells the chromosomes are only slightly elongated, or may be nearly globular (figs. 77, 80, 82). No good examples were found of the groups of daughter chromosomes when first arrived at the poles. Slightly later, when the daughter (spermatozoid) nuclei have been organized, the chromatin is found in rather numerous, scattered roughish angular grains, arranged in a rather coarse reticulum (figs. 55*a*, 83, 84). At about this time or soon after, the chromatin granules may collect into (about ten) evidently composite masses of rounded form (figs. 55*a*, *b*, 81, 83). In fig. 85 the size and number of granules apparently "chromomeres" in each mass or chromosome are approximately constant, like the number of the chromosomes themselves.

From the time of separation of the young androcytes to the formation of a cell plate (but not of a definite cell wall) they are commonly flattened against each other, so as to look somewhat triangular in cross-section, that is, in a section parallel to the axis of the last spindle (fig. 86). This form may sometimes be due to the diagonal division of the cubical mother cell, but in other cases apparently to the subsequent flattening of the daughter cells against each other. The spermatid nucleus is at first globular and central in position, as is true of *Blasia* (SHARP 11), *Polytrichum* (ALLEN 1), and many other archegoniates. The chromatin granules are rather large and only sparsely connected into a reticulum. Later the nucleus begins to elongate, and the chromatin grains become connected up more abundantly, often in somewhat parallel beadlike chains (figs. 64, 87, 92).

It is at this stage of nuclear elongation also that the blepharoplast for the first time becomes clearly evident, as a darkly staining rod on one side of the spermatid, beside the elongating nucleus (figs. 57, 86, 93). The blepharoplast of *Plagiochila* seems usually to be in actual contact with the nucleus from the beginning of elongation of the latter. Only rarely can it be seen to lie nearer the cell wall and to separate from the nucleus (fig. 97*a*), as it is shown to do in *Marchantia* (IKENO 6), *Blasia* (SHARP 11), and *Polytrichum* (ALLEN 1). Earlier than this stage nothing can be found constantly in the cell that can be identified certainly as the progenitor of this dark rod which is later to bear the two cilia of the mature spermatozoid. A blepharoplast then does not become unmistakably evident at so early a stage here as IKENO, WOODBURN, ALLEN (1), and SHARP (11) have found it to do in other bryophytes. There is thus no evidence that the blepharoplast is derived from a persistent body having the characteristics of a centrosome.

In the later development of the spermatozoid the nucleus continues to elongate, usually becoming more slender at one end than at the other, and the whole nucleus thus comes to have a rather pear-shaped form (figs. 58, 65, 94*d*). This is apparently the characteristic manner of elongation of the spermatozoid nucleus in both bryophytes and pteridophytes (SHAW 12, IKENO 6, YAMANOUCHI 15, ALLEN 1, SHARP 11). During these phases of development the chromatin granules of the nucleus often take a somewhat indistinctly beadlike arrangement, with the strings longitudinal to the nucleus (figs. 65*b*, 92).

When the spermatozoid has later become elongated to a length four or five times its diameter, the chromatin often has the form of six or seven transverse groups or bands (figs. 59, 60, 95*d*). Sometimes, although this is not always clear, these bands have a slant which suggests that one is really seeing successive turns of a single spiral which appears to reach nearly the whole length of the spermatozoid (figs. 61, 67, 95). Fig. 60*b*, of a cross-section of such a nucleus, shows that the chromatin band is peripheral and not complete in any one plane. In fig. 94*a*, which is that of a well advanced spermatozoid, only the barest suggestion of transverse bands of the chromatin is evident. In some few young spermatozoids of about this age, which were evidently well fixed, the whole nucleus seemed coiled (figs. 66, 95*c*, 96). In all cases where the relative ages could be determined these coiled nuclei occurred at an earlier stage of development than the characteristic coiling of the whole spermatozoid in a single plane, which is shown in figs. 61, 88, 90, 94, 98. In some of these cases of the apparent coiling of the nucleus within the uncoiled cytoplast it seemed possible that the apparent coiling was due to the alternation of the bulk of the chromatin on the two sides of the elongated nucleus. In other cases, in slides where adjoining antheridia were well fixed, the independent interpretation of several trained observers agreed in regarding the whole nucleus as really spirally coiled, as is indicated in figs. 66 and 96. No adequate series of consecutive later stages was found, in spikes where this type of coiling of the nucleus occurred, that would allow following closely the further development of this sort of spermatozoid. This coiling may prove to be abnormal although it is not rare. It is hoped that further study of this and other species of liverworts will show its significance.

The further flat (watch-springlike) coiling of the spermatozoid nucleus seems to be accompanied by a gradual separation and disappearance of the transverse chromatin bands. This may be due primarily to a rearrangement of the chromatin granules composing the bands (figs. 67*b*, 90, 94*a*, *d*). As is evident from figs. 88, 90, 95*a*, *b*, however, the chromatin still retains its peripheral position in the nucleus. The chromatin of the elongated spermatozoid nucleus seems to form a sort of curved, tubular reticulum, along the outer side of which lies the blepharoplast. This peripheral position of the chromatin shows especially clearly in all moderately stained and lightly stained cross-sections of spermatozoids, from this stage up to the practically ripe ones (figs. 89, 94*b*, 99).[2]

The darkly staining blepharoplast that first becomes distinguishable when the spermatozoid nucleus begins to elongate, remains clearly distinct in most spermatozoids from this time until the spermatozoid is mature. At first the blepharoplast is rather short and thick, one end being thicker than the other, and is but slightly curved (fig. 93). A spherical blepharoplast has not yet been found, unless the dot near the upper pole of the spindle in figs. 80 and 81 be so interpreted. Later the blepharoplast is attenuated to a length many times its diameter, and becomes curved to conform to the coiled spermatozoid, along the slender anterior end of which it lies (figs. 88; 94, 97). In most cases the blepharoplast seems to lie in close contact with the nucleus. In fig. 97*a* is shown one of the few cases where it is evidently separate throughout most of its length. With increasing attenuation the blepharoplast commonly remains practically homogeneous, although it may occasionally show slight irregularities in thickness (figs. 88, 92). It seems to be always continuous and never vacuolated or clearly fragmented, however, as SHARP (11) has shown it to be in *Blasia*, and as it had earlier been found to be in certain pteridophytes (SHAW 12) and cycads (CHAMBERLAIN 3). The appearance shown in fig. 90, where the coiled spermatozoid has a beadlike outer border, seems clearly due to the granules of peripheral chromatin and not to any discernible fragmentation of the blepharoplast.

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[2] It was evidently a series of peripheral chromatin granules similar to those referred to previously, which were seen in cross-section of the spermatozoid of *Monoclea*, complicated perhaps by the overlapping coils of the two spermatozoids of each pair, that led the writer (7) to a misinterpretation of this stage of the spermatozoid, in an earlier study of that liverwort. A reexamination of the slides shows that these stages of the spermatozoid may there be interpreted as they have been here in *Plagiochila*.

The ripe spermatozoid has a slender body, cylindrical except for its tapering anterior fifth. It is coiled to about one and a-half or one and two-thirds turns in a flat spiral. Its two cilia are about one-third the length of the body itself. The cytoplasm of the body of the spermatozoid is but slightly granular, while that of the rest of the androcyte shows no appreciable differentiation, except usually a rather globular, granular mass (limosphere?) near the center of the coil (figs. 98, 99). When the living mature spermatozoids are discharged into surrounding water drops by the irregular bursting of the wall of the antheridium (fig. 35), they are almost transparent. As the spermatozoid swims about, its flat spiral commonly becomes drawn out to a rather corkscrew-like form. The entrance of the spermatozoid to the archegonium and the actual fertilization of the egg formed in the latter organ have not yet been observed. It is expected that such stages of the development of the sporogonium as have been seen will be described in a later paper, when sporogenesis has been studied more completely.

Summary and conclusions

1. This study was undertaken primarily to discover the formation and detailed structure of the very striking antheridial spikes of this liverwort, the development of antheridium and spermatozoids, the origin and organization of the blepharoplast, and the occurrence or non-occurrence of sex chromosomes.

2. The antheridial spike consists of 20–100 diminutive and strikingly urn-shaped fertile leaves. These are sometimes in one continuous series, but their development is often interruped by the appearance of one or more series of larger sterile leaves. The spike may be simple or it may fork once or several times to form a dorsiventral, fanlike spray. The spike may persist and grow for several years. At any one time it may contain up to twenty-two living antheridia, from one of but a few cells to those holding ripe spermatozoids.

3. The development of the individual antheridium in this species resembles that in *Plagiochila asplenoides*, so far as this latter was followed by LEITGEB.

4. The nucleus of each of the spermatozoids, of which there are 25,000 or more in each antheridium, is at first globular, then pear-shaped, and finally becomes a slender cylinder or club. It has a peripheral, granular net or sometimes a series of transverse bands of chromatin. The young chromosomes, at early prophase of the last division, show series of minute component granules, the chromomeres, as do the chromosomes of the nuclei of the young spermatozoids organized immediately after this division.

5. No constant difference in form or size could be discovered among the chromosomes of the same or of different spermatozoids. Comparison of mitoses in male and female plants gave, likewise, no evidence of the presence of sex chromosomes here.

6. The blepharoplast first becomes clearly evident as a short rod when the nucleus of the young spermatozoid begins to elongate. It could not be constantly found at the poles of the last mitotic spindle. It was never seen in earlier mitoses. No clear case of vacuolization and fragmentation of the blepharoplast could be found.

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EXPLANATION OF PLATES I–III[3]

PLATE I

Fig. 5.—Horizontal section of apex of vegetative branch, showing initial cell, younger segments, and bases of leaves of two lateral ranks; above initial are transverse sections of trichomes arising from its ventral segments; ×130.

Fig. 6.—Approximately horizontal section of vegetative growing point, showing a branch rudiment at right; ×240.

Fig. 7.—Dorsal view of young leaf; ×240.

Fig. 8.—Approximately sagittal section of tip of young antheridial spike, seen from right side, showing apical cell and three young antheridia; ×240.

Fig. 9.—One-celled rudiment of antheridium with subtending leaf, in horizontal section of spike; ×240.

Fig. 10.—Part of approximately sagittal section of antheridial spike, showing section of involucral leaf inclosing young antheridium, and basal end of next younger involucral leaf; ×240.

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[3] All photographs and drawings made by the writer.

FIG. 11.—Part of approximately sagittal section of spike, showing young antheridium, with wall and spermatogenous cells defined, and inclosing involucral leaf; ×240.

FIG. 12.—Part of obliquely sagittal section of two successive involucral leaves, showing inclosed antheridia at about the level of antheridia 1 and 2 in fig. 36; antheridium at left is cut through adaxial half; ×240.

FIG. 13.[4]—Nearly transverse section of apex of young antheridial spike, showing stem initial, younger segments, form of involucral leaves and positions of inclosed antheridia; antheridia shown in dotted lines are at levels of spike below that of section from which rest of figure is drawn; ×130.

FIG. 14.—Part of approximately transverse section of sterile stem apex, showing initial with youngest ventral segment and trichome arising from next older ventral segment; ×160.

FIG. 15.—Adjoining section to that shown in fig. 14, showing base of trichome referred to; ×160.

FIG. 16.—Transverse section of antheridial spike below apex, showing stem, attachment of involucral leaves to latter, antheridial stalks in youngest involucres, and positions of older antheridia in outer involucres; ×30.

FIG. 17.—Transverse section near middle of mature antheridial spike, showing stem and completely closed basal portions of four successive involucral leaves, with transverse sections of two antheridial stalks; note absence of any broad ventral flap from basal portion of involucral leaf; ×30.

FIG. 18.—Transverse section of antheridial spike through two fullgrown antheridia, showing structure of stem and attachment of involucral leaves to latter; ×30.

FIG. 19.—Part of section similar to that shown in fig. 18, enlarged to show details, including thickening of outer cell walls of stem and involucre; ×130.

FIG. 20.—Transverse section of stem and single involucral leaf which, as occasionally happens, incloses two antheridia, the inner and upper being the younger; ×260.

FIG. 21.—Chloroplasts from leaves fixed in Flemming's solution; ×1340.

FIG. 22.—Horizontal section of young antheridium, showing stalk and upper surface of eight-celled body; ×240.

FIG. 23.—Lateral view of young antheridium, showing cells of abaxial surface; ×240.

FIG. 24.—Horizontal section of young antheridium, showing 2-rowed stalk, wall, and one of the two primary spermatogenous cells present at this stage; ×240.

FIG. 25.—Part of approximately horizontal section of spike, showing antheridium with four spermatogenous cells and stalk two cells wide; ×240.

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[4] Figs. 13–19 and 25–27 are from slides prepared by Dr. B. H GRAVE when he was a student at Johns Hopkins University

FIG. 26.—Approximately sagittal section of antheridium from right side of spike, showing four of its eight spermatogenous cells; ×240.

FIG. 27.—Surface view of young antheridium from horizontal section of spike, showing arrangement of cells in wall; cf. fig. 28; ×250.

FIG. 28.—Surface view of young antheridium of *Plagiochila asplenoides*;[5] compare plan of arrangement of surface cells with that in fig. 27; ×250.

FIG. 29.—Lateral view of abaxial surface of young antheridium, showing arrangement of cells of wall; ×250.

FIG. 30.—Sagittal section of antheridium shown in fig. 29, showing wall and five of the ten spermatogenous cells; ×250.

FIG. 31.—Approximately sagittal section of older antheridium, showing positions of octant walls in spermatogenous group of cells; ×240.

PLATE II

FIG. 32.—Sagittal section of two-thirds grown antheridium, showing single row of stalk cells, wall, and spermatogenous cells (androgones); ×350.

FIG. 33.—*a*, sagittal section of fullgrown antheridium after formation of androcyte nuclei, showing over 1000 cells in longitudinal section; *b*, cross-section of stalk of fullgrown antheridium; ×220.

FIG. 34.—Lateral surface of mature antheridium, showing size and form of surface cells; from alcoholic specimen; ×125.

FIG. 35.—Lateral view of ruptured, living, ripe antheridium; ×125.

FIG. 36.—Horizontal section of forked antheridial spike, showing relative size of successive antheridia and position of involucral leaves; branch of spike (dotted lines) is from third section beyond that from which rest of figure is drawn. Two developing eggs (perhaps of tardigrade or nematode) lie beside stalk of antheridium no. 8; lowest involucral leaf incloses two antheridia; ×25.

FIG. 37.—Part of radial longitudinal section of axis of mature spike, showing character of thickened surface cells and thinner-walled axial cells of stem, also bases of antheridial stalk and involucre; ×170.

FIG. 38.—Transverse section of body of very young antheridium, showing median longitudinal wall which most frequently stands perpendicular to radius of stem passing through stalk as it does here; ×350.

FIGS. 39–42.—Transverse sections of body of antheridium, successive stages, showing sequence of cell walls which delimit spermatogenous cells from antheridial wall; arrow indicates direction of axis of spike from antheridium, and shows position of median wall in relation to sagittal plane; ×350.

FIG. 43.—View of top of young antheridium, showing positions of median and first quadrant walls; ×350.

FIG. 44.—Transverse section through base of antheridium, showing median wall and next following anticline in each half; ×350.

FIG. 45.—Transverse section of stalk of young antheridium; ×350.

FIG. 46.—Transverse section of stalk of ripe antheridium; ×350.

[5] Traced from LEITGEB, Vergleichende Untersuchungen. Heft II, Tav X, fig. 21.

FIG. 47.—Transverse section of two antheridia in same involucre; arrow indicates direction of axis; note that lower antheridium is further developed than upper (the next younger antheridium decidedly less developed than either of these); another involucre of this same spike also bore two antheridia; ×350.

FIG. 48.—Surface view of top of antheridium at age of that shown in fig. 32; ×350.

PLATE III

FIG. 49.—Transverse section of antheridium about age of that shown in fig. 32, showing four of the eight octants of spermatogenous cells; ×350.

FIG. 50.—Part of approximately sagittal section of antheridial spike, showing relative sizes and positions of eleven successive antheridia on one side of axis, to serve as index of ages of antheridia and spermatogenous cells shown in figs. 51–61; ×40.

FIG. 51.—Longitudinal section of very young antheridium (no. 1 of fig. 50) and its involucre; ×750.

FIG. 52.—Section (sagittal to spike) of antheridium no. 2 of fig. 50, showing stage of development and structure of nuclei; ×750.

FIG. 53.—Sagittal section of young antheridium (no. 3 of fig. 50); ×750.

FIG. 54.—Part of sagittal section of halfgrown antheridium, showing single wall cell and two spermatogenous cells (no. 4 of fig. 50); ×1400.

FIG. 55.—*a*, nucleus of androcyte reorganizing after last nuclear division (polar view) from antheridium no. 5 of fig. 50; ×1700. *b*, telophase from same antheridium but slightly more advanced than that in *a*, showing two sister androcyte nuclei (no. 5 of fig. 50); ×1400. *c*, later telophase of last nuclear division (no. 5 of fig. 50), showing chromatin net forming in androcyte nuclei; ×1700.

FIG. 56.—Young spermatozoids from antheridium no. 6 of fig. 50, showing nuclei slightly elongated (axis of last spindle in plane of page; that is, spermatozoids seen from edge; ×1400.

FIG. 57.—Similar view of two slightly older spermatozoids from antheridium no. 7 of fig. 50; ×1400.

FIG. 58.—*a*, spermatozoids from antheridium no. 8 of fig. 50 seen from edge; nuclei and blepharoplasts now somewhat elongated and bent; *b*, spermatozoid of same age and from same antheridium as those in *a*, seen from side of incipient coil instead of from edge; nucleolus no longer evident; chromatin in large granules; ×1400.

FIG. 59.—Still older spermatozoid from antheridium no. 9 of fig. 50 seen from edge, and showing greater elongation, with chromatin granules in transverse bands; ×1400.

FIG. 60.—*a*, two spermatozoids from antheridium no. 10 of fig. 50 seen from edge, showing but slight advance in development over that in fig. 59; *b*, transverse sections of two spermatozoids of same antheridium, showing peripheral position of chromatin in each band; ×1400.

FIG. 61.—Spermatozoid from antheridium no. 11 of fig. 50 seen partly from edge, showing slender spindle-like form; ×1400.

FIG. 62.—Spermatogenous cells from young antheridium (about age of no. 4 in fig. 50), showing organization of nucleus, with chromatin, nucleolus, and one or two other darkly staining bodies; ×1700.

FIG. 63.—Spermatogenous cell from young antheridium slightly older than that in fig. 54, showing organization of nucleus and cytoplasm; ×1700.

FIG. 64.—Young spermatozoid seen from edge (about age of those in fig. 57); ×1600.

FIG. 65.—Three young spermatozoids from antheridium no. 7 of slide 13: *a*, seen from edge of coil; *b*, *c*, seen more from the side, showing beginning of coiling; ×1600.

FIG. 66.—Two spermatozoids from antheridium no. 8 of slide 13, slightly older than that drawn in fig. 65, showing frequent arrangement of chromatin that gives spermatozoid nucleus the appearance of being spirally coiled; this spiral form not always distinguishable in spermatozoids between those of ages shown in figs. 65 and 67; ×1600.

FIG. 67.—Two pairs of spermatozoids from antheridium no. 9 of slide 13: *a* slight coiling; *b*, spindle-like form and aggregation of chromatin into bands; blepharoplasts not evident; ×1600.

FIG. 68.—Spermatozoid showing 1½ coils, from antheridium no. 10 of slide 13, with flagella(?) extending forward from tip of spermatozoid; ×1600.

FIG. 69.—Androcyte mother cell, showing chromatin net and distinct nucleolus (phase following that shown in fig. 54); ×1600.

FIG. 70.—Last androgone (androcyte mother cell), showing structure of nucleus just before initiation of last division (that which forms spermatozoid nuclei); ×1600.

FIG. 71.—Nucleus of androcyte mother cell showing late prophase of last mitosis; this and figs. 72–84, except 75, are from a single antheridium, and show range in stages of development that may occur in different parts of same antheridium); ×1600.

FIG. 72.—Prophase of last mitosis in antheridium, showing long rods and loops of rather deeply stained chromatin; ×1600.

FIG. 73.—Group of three adjacent nuclei, from same antheridium as that in fig. 71, showing elongated and bent chromosomes, and something of the transverse bandlike localization of chromatin granules; ×1600.

FIG. 74.—*a*, another nucleus from same antheridium as that from which figs. 71 and 72 were taken, showing more striking transverse banding of chromosomes; ×1600. *b*, two chromosomes of age shown in *a*, showing transverse banding in more detail; ×1750.

FIG. 75.—Nucleus (densely stained) between stages shown in figs. 74 and 76 (from different spike); ×1600.

FIG. 76.—Nucleus of androcyte mother cell in prophase, slightly more advanced than those in figs. 71–73, showing chromosomes more condensed but chromatin granules still peripheral in position; ×1600.

FIG. 77.—Two nuclei slightly more advanced than those shown in figs. 74 and 76; chromatin of the ten chromosomes more condensed and deeply stained; ×1700.

FIG. 78.—Late prophase of last mitosis in androcyte mother cell, showing chromatin thread broken to short thick chromosomes, with chromatin aggregated chiefly at surface; ×1600.

FIG. 79.—Still another nucleus of same antheridium as that used for fig. 78, showing contraction of chromosomes; most of chromatin still peripheral in position in each; ×1600.

FIG. 80.—Metaphase of last division in antheridium, showing chromosomes, spindle, and blepharoplast(?) at upper pole; ×1600.

FIG. 81.—Telophase of last mitosis in spermatogenous cell (androcyte mother cell), showing first step in organization of the two resulting spermatozoid nuclei; nucleus from same antheridium as that from which the last five figures were drawn; ×1600.

FIG. 82.—Metaphase of last division of androgone nucleus with blepharoplast (?) near upper pole; ×1600.

FIG. 83.—Late telophase of division of androcyte mother cell nucleus, showing chromatin granules in approximately ten groups at each pole, precise number of groups not determined; ×1400.

FIG. 84.—Polar view of one daughter nucleus of age shown in fig. 83; ×1400.

FIG. 85.—Polar view of nucleus of young spermatozoid of slightly later stage than those shown in figs. 81 and 83, although drawn from same antheridium with these and figs. 71–74 and 76–80, showing chromosomes with component granules (chromomeres); ×1700.

FIG. 86.—Two young spermatozoids showing triangular shape, in section parallel to last spindle; nuclei still nearly spherical, with chromatin grains distinct; ×1400.

FIG. 87.—Spermatozoid of next older antheridium of spike from which fig. 86 was drawn, showing slight elongation of nucleus and faint indication of longitudinal chains of chromatin granules; ×1600.

FIG. 88.—Young spermatozoid from antheridium next older than that in fig. 87, showing elongation of nucleus and blepharoplast and suggestion of transverse bands of chromatin; ×1300.

FIG. 89.—Two young spermatozoids, adjoining that figured in fig. 88, showing transverse sections of spermatozoid nuclei and peripheral position of chromatin granules; ×1600.

FIG. 90.—Spermatozoid from same antheridium as that used in fig. 88, showing slightly greater coiling, attenuation of anterior end of nucleus, and peripheral arrangement of chromatin granules; ×1600.

FIG. 91.—This and five following figures are from six successive antheridia

on same side of single spike; fig. 91 itself is of a very young spermatozoid from antheridium no. 1 of this spike, showing still globular nucleus with small and definite number of chromatin bodies; × 1300.

FIG. 92.—Two young spermatozoids from antheridium no. 3 of this spike, showing flattened sides and few large chromatin grains; ×1300.

FIG. 93.—*a*, *b*, Three young spermatozoids from antheridium no. 3 of this spike, showing blepharoplast beginning to elongate, while nucleus is still globular; ×1300.

FIG. 94.—*a-d*, Five spermatozoids from antheridium no. 4 of this spike: *a* from side, *b* in cross-section, *c* and *d* from edge; ×1300.

FIG. 95.—*a-d*, Four spermatozoids from different parts of one antheridium, no. 5 of this spike, showing further elongation and coiling, and peripheral position of chromatin. This latter often appears in five or six transverse bands (or turns of spiral) which in cross-section seem incomplete, as in same stage shown in fig. 60 *b*. In *c* the whole body of spermatozoid nucleus appears coiled nearly twice around like a corkscrew. This slide, rather faintly stained, showed no clear blepharoplast, cf. fig. 66; ×2600.

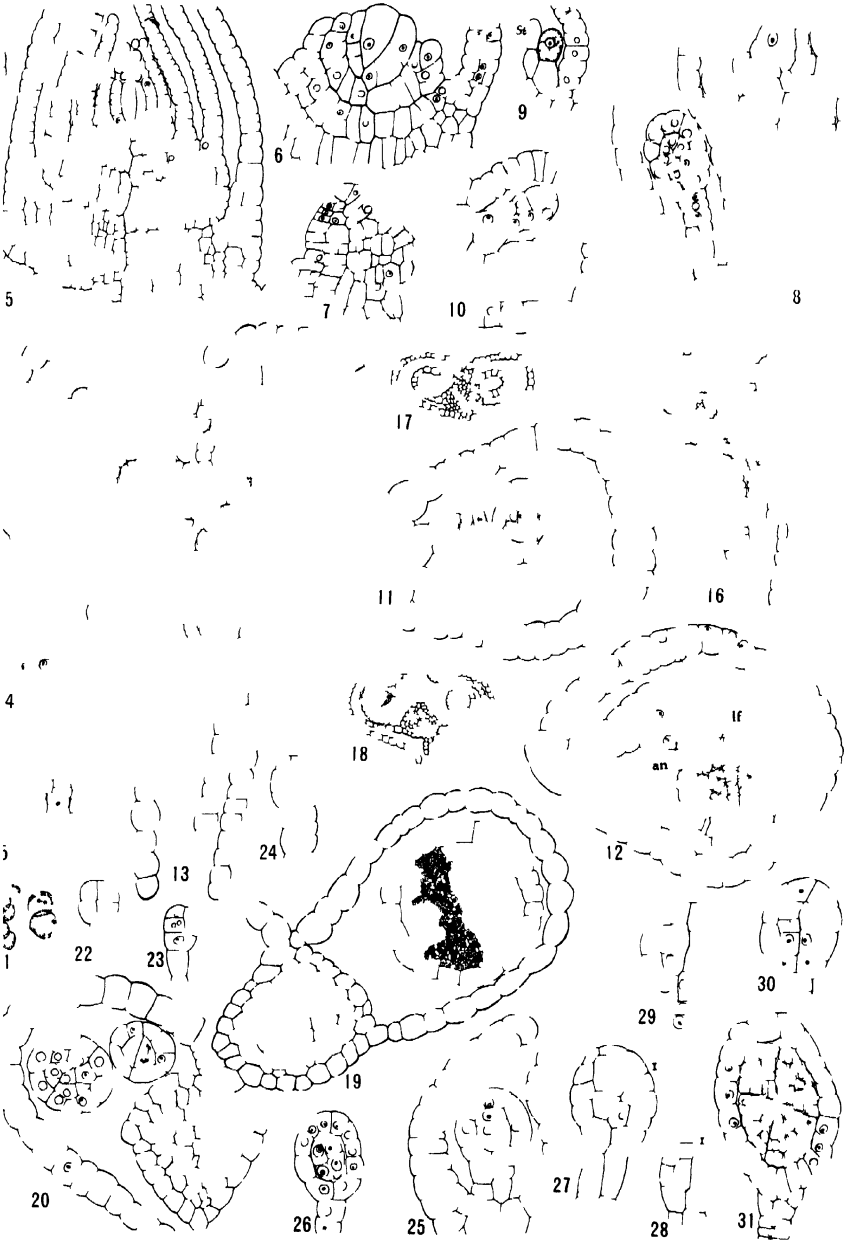
FIG. 96.—Spermatozoid from antheridium no. 6 of this series, showing marked coiling of nucleus; blepharoplast not evident; ×1300.

FIG. 97.—*a*, *b*, Three young spermatozoids from antheridium no. 7 of its spike, about age of that shown in fig. 94, showing distinct blepharoplast which is clearly free from nucleus; ×1300.

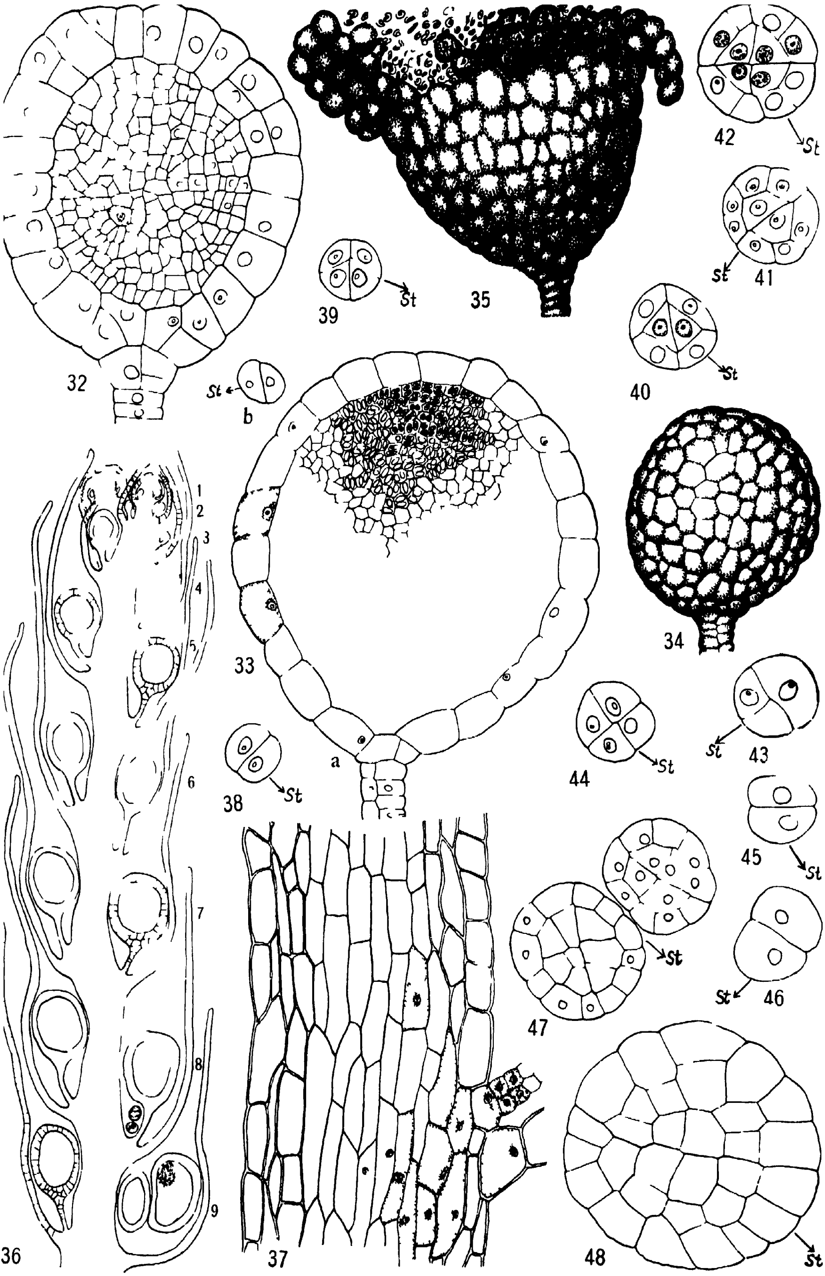
FIG. 98.—Nearly mature spermatozoid from antheridium no. 5 of same spike from which fig. 99 was drawn; ×1400.

FIG. 99.—Optical transverse sections of two coiled spermatozoids of same antheridium as those drawn in fig. 98, showing limosphere(?) and tubelike arrangement of chromatin granules; ×1700.

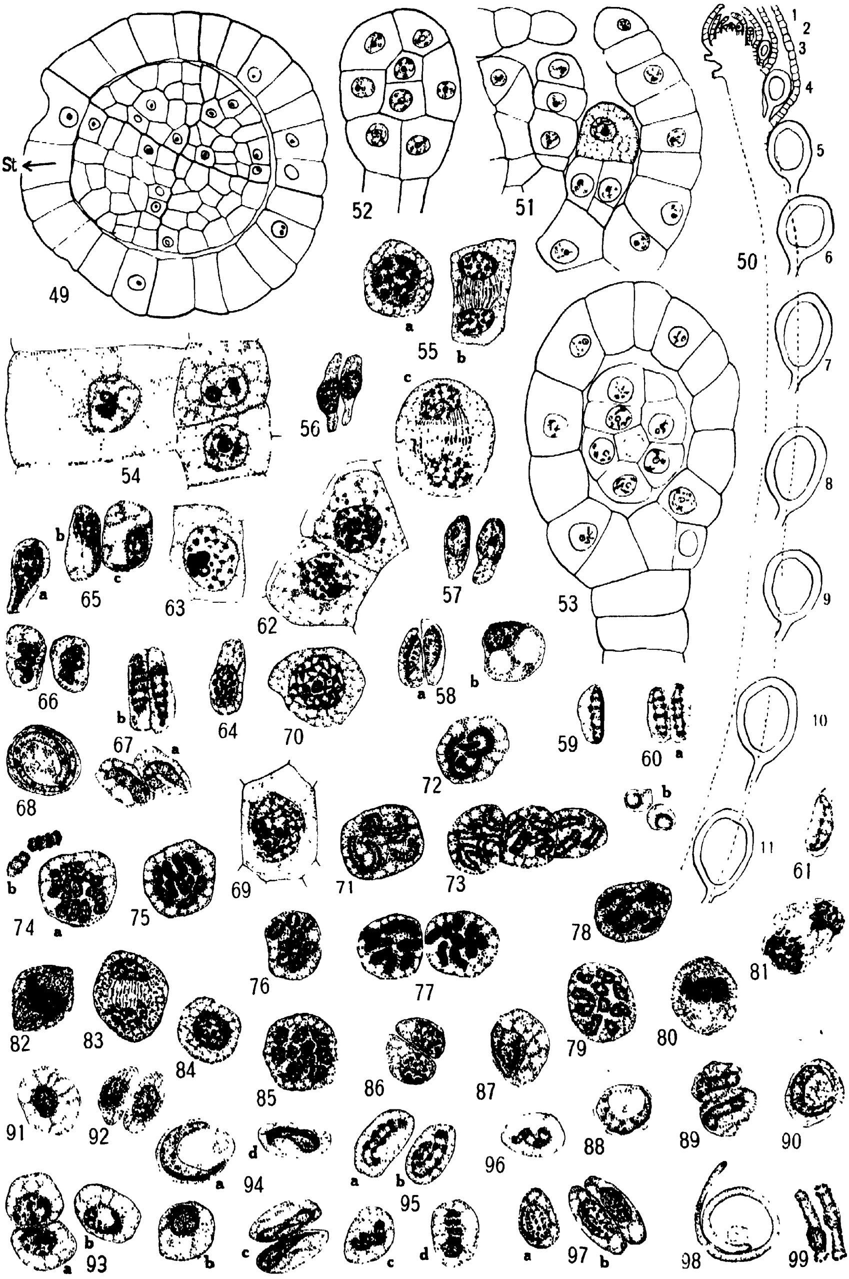
PLATE I



JOHNSON on PLAGIOCHILA



JOHNSON on PLAGIOCHILA



JOHNSON on PLAGIOCHILA

MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE[1]

II PANICEAE AND ANDROPOGONEAE

George L Church

(WITH PLATES IV–VI)

In the first part of this study,[2] the importance of hybridization in the multiplication of species in the larger families and genera of plants, together with the substantiating cytological evidence, was reviewed The cytology of the maturation of the pollen grains in thirteen species of grasses, representing the tribes Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae, was reported, with an account of the methods employed in the technique of the investigation The species *Phalaris arundinacea* L is a normal diploid, the other species are considered to have arisen by means of hybridization because of the evidence of polyploidy and abnormal cytological behavior

In this article, the cytology of eighteen species and varieties representing the tribes Paniceae and Andropogoneae is described Because of the notorious variability in the dichotomum type of *Panicum*, most of the investigation in the genus has been focused on this section

Cytology of species

PANICEAE

Digitaria sanguinalis (L) Scop , naturalized weed from Europe, pollen 30–40 per cent imperfect —This species is tetraploid, showing 14 ringed bivalents at diakinesis Cytomyxis, occurring at this stage, is believed to be the cause of the masses of chromatin often seen in the cytoplasm during the heterotypic division Bivalent laggards are frequently seen in this first division, and may be extruded during the metaphase, which is very tardy in forming the plate Homeotypic divisions are normal, but polycary is occasionally found.

Paspalum muhlenbergii Nash, pollen 30 per cent imperfect.—

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[1] Contribution from the Laboratories of Plant Morphology, Harvard University

[2] Bot Gaz 87. 608–629 1929

This species seems to be a diploid in the series in which 10 rather than 7 is the haploid number. The chromosomes here appear small and spherical as they do in *Panicum*. Whereas 10 bivalents usually appear at diakinesis, counts of 9 may be made definitely in many instances. Counts of metaphase plates of the heterotypic division show a similar aberration, even a few instances of 11 bivalents being noticed. Here again cytomyxis occurs to a considerable degree during the spireme stage, often persisting through diakinesis. Many of the latter stages may be found with chromatin disintegrating near large vacuoles in the peripheral cytoplasm of the mother cell. The heterotypic division is marked by many lagging bivalents in the metaphase, a large percentage of which are extruded. Anaphase laggards are less frequently left on the spindle at telophase. Cytoplasmic strands may be seen between mother cells at interkinesis; consequently the diad stage is seen presenting at times a polycaric appearance. The chromosomes are rather minute in the homeotypic division, but the telophase may be found with added extruded pieces of chromatin. Polycary, as it not infrequently occurs in the tetrad stage, is therefore to be expected.

PANICUM

DICHOTOMIFLORA

Panicum dichotomiflorum Michx.; pollen 20–30 per cent imperfect.—Counts of metaphase plates of the heterotypic division reveal 27 bivalent chromosomes, making this species hexaploid, reckoning 9 as the basic haploid number. Occasionally metaphases and anaphases of the first division present a considerable number of laggards, and extrusions on the spindle at the telophase are seen to follow as a result. The homeotypic divisions are quite regular, however, and very seldom does polycary occur. Cytomyxis is frequently observed at the spireme stage and at interkinesis.

CAPILLARIA

Panicum miliaceum L., cultivated in and advanced from the Old World; pollen 10–20 per cent imperfect.—Polar views of metaphase plates of the heterotypic division usually show 20 bivalents, although many definite counts of 18 have been made. This is doubtless a tetraploid species originally having 10 as the basic haploid number, in common with such genera as *Paspalum*, *Sorghastrum*, and *Zea*. Occasional extrusions have been noted at the heterotypic metaphase together with lagging in the anaphase. Extranuclear chromatin may appear in the telophase as a consequence. The homeotypic metaphase often presents laggards, and polar views of regular spindles at times present only 18 chromosomes. The anaphase is quite regular, however. Cytomyxis has been observed at interkinesis, but no polycary has been found.

DICHANTHELIUM[3]

LANUGINOSA

Panicum lindheimeri Nash.—*P. huachucae*, *P. tennesseense*, and *P. implicatum* have for the most part been treated as varieties of the preceding species by FERNALD (11). Pollen is completely sterile in the species and its varieties.

Panicum lindheimeri Nash var. *typicum* Fern.—Diakinesis (fig. 44) usually reveals 9 bivalents, one or two pairs very loosely (if at all) united. Cytoplasmic chromatin may also be observed at this stage. Such extrusions are unquestionably due to the large amount of cytomyxis occurring at the spireme stage, and at times persisting through the diakinesis. Frequently whole anthers display cytomyxis to such an extent that it is doubtful whether any of the mother cells develop farther than the prophase. Lagging and extrusions are common phenomena throughout all the divisions. The early heterotypic anaphase (fig. 45) is marked by a very uneven separation of the bivalents, resulting in lagging in the later stage (fig. 46). Such laggards may persist as extrusions in the early diad stage (fig. 47). Figs. 50, 51, and 54 illustrate the striking irregularity of the homeotypic division. An occasional instance (fig. 53) may be found where apparently the homeotypic spindle does not form, but the chromatin is simply pulled into each half of the dividing cell, the split being initiated at the equatorial region in usual fashion. It is difficult to judge the degree to which such amitotically dividing cells may mature, since practically all of the pollen collapses. A difference in the number of chromosomes seems to exist in this species. Homeotypic equatorial plates (fig. 52) often show only 8 bivalents, while heterotypic plates (fig. 49) usually show 9. A loss of a bivalent as an extrusion could explain this discrepancy, yet counts of 8 have been made at diakinesis and followed throughout the succeeding division. Polycary is consistently displayed in the tetrads and not infrequently polyspory to the extent of a small additional pollen grain (fig. 55). Homeotypic telophases often display much extruded chromatin. Fig. 56 shows a typically shriveled mature grain.

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[3] Subgenus of which *P. dichotomum* is the type, cf HITCHCOCK and CHASE 23. Examination in all cases is of the spring florets.

Panicum lindheimeri Nash var. *septentrionale* Fern.—The same count of 9 bivalents is made clearly in diakinesis. This form revealed, however, much fewer irregularities than any of the dichotomum-like species of *Panicum* investigated. A few laggards were found at times in the heterotypic anaphase, and a slight degree of polycary was displayed in the tetrads. All other stages appeared quite normal.

Panicum lindheimeri Nash var. *fasciculatum* (Torr.) Fern.—Here again diakinesis shows 9 bivalents, many being very loosely paired. Cytomyxis in the prophase stages produces cytoplasmic chromatin that often persists through the diad stage. Lagging is common in the heterotypic divisions (figs. 57, 58). Instances of a collapsed condition of the heterotypic spindle are illustrated in figs. 59 and 60. Non-disjunction accompanied by lagging may give rise to a different assortment of chromosomes at the poles (fig. 60). Fig. 61 represents an unusually early occurring interkinesis (before completion of the diad split), in which a decided difference in the number of chromosomes in each nucleus is easily observed. The diad stage is commonly marked by extruded chromatin. Homeotypic divisions present lagging chromosomes only occasionally, but polycary is often found in the tetrads.

Panicum lindheimeri var. *implicatum* (Torr.) Fern.—In the degree to which the four forms of *P. lindheimeri* present irregularities, this variety ranks third, the varieties *typicum*, *fasciculatum*, and *septentrionale* ranking first, second, and fourth respectively. Cytomyxis in the prophase stages is observed only occasionally. Lagging and extrusions still characterize the majority of the heterotypic divisions however, and polycary is often found. The material furnished an abundance of examples of the heterotypic telophase displaying laggards on the spindle obviously excluded from the nuclei. The homeotypic divisions, so far as observed, proceed rather normally.

Panicum subvillosum Ashe; pollen 70–80 per cent imperfect.—Nine is here the haploid number, but frequent deviations from this are encountered. Eight is a common count, 7 and 10 being occasional. Two or three pairs of chromosomes often are very loosely (if at all) joined in diakinesis, and they persist in this condition, usually as laggards, on the heterotypic spindle. Such univalents may be extruded as the spindle of the first division is forming; consequently, in making chromosome counts, great care was exercized to distinguish bivalents and univalents, and to determine the number in both polar and spindle views of both divisions. Cytomyxis is very common, even in the diakinesis, 3 or 4 cells being involved simultaneously and whole chromosomes passing between them. Laggards and extrusions abound in the reduction division. Late anaphases have been observed with 9 chromosomes at one pole and 7 at the other. Homeotypic divisions are typified by lagging only to a slighter degree. Polycary is common, the extra nuclei often assuming proportions of one-half to two-thirds the diameter of the normal nucleus of each member of the tetrad.

COLUMBIANA

Panicum tsugetorum Nash; pollen 90 per cent imperfect.—Nine holds fairly consistently as the haploid number of chromosomes. Conditions of irregularity differ only slightly in degree of occurrence from those mentioned in the preceding species, *P. subvillosum*. Metaphases of both divisions assemble very tardily. Extrusions occur for the most part in the first division, and are very evident in the diad stage and later in the tetrads (polycary).

SPHAEROCARPA

Panicum sphaerocarpon Ell.; pollen 80–90 per cent imperfect.—Typical cytological irregularities of the genus are illustrated in this species. Fig. 62 shows a diakinesis with 9 bivalents, several only loosely associated and probably a portion of one pair extruded. Fig. 63 shows the heterotypic metaphase with nearly half the complement of bivalents lagging at one pole of the spindle. Unequal numerical distribution of the disjoined mates is illustrated in the anaphase (fig. 64). Extruded laggards and cytomyxis may be observed in the telophase (fig. 65). The homeotypic divisions (figs. 66, 68) again present laggards and extrusions from the heterotypic. Varying counts made of metaphase plates (fig. 67) may undoubtedly be attributed to the unequal segregation noticed in the first division. Polycary is frequently observed.

OLIGOSANTHIA

Panicum scribnerianum Nash; pollen 70–80 per cent imperfect.—An abundant amount of cytomyxis at diakinesis (fig. 69) is seen in this species. This illustration shows the stretched chromatin between the nuclei and the transference of whole chromosomes and even nucleoli to the cytoplasm of adjacent mother cells. This phenomenon may persist even up to the stage when the nucleolus has disappeared and the spindle is being initiated (fig. 70). Such a mother cell as the upper one of the two shown in fig. 70, with all of its chromatin gone, naturally disintegrates. Extruded chromatin, resulting from disruption of nuclei suffered during cytomyxis, persists in the metaphase of the first division (fig. 71). This figure also shows non-paired or loosely paired mates on the heterotypic spindle. The anaphase displays typical lagging (fig. 72). By the time the homeotypic division is reached, a decrease in the chromosome complement from the basic haploid number of 9 is often observed. This division is further marked by the extrusion of whole chromosomes and fragments (figs. 73, 74). As would be expected, polycary is common in the tetrads (fig. 75).

Echinochloa crus-galli (L.) Beauv., naturalized from Europe. This form corresponds closely to the variety *longiseta* of WIEGAND (48); pollen 10–20 per cent imperfect.—Diakinesis reveals 21 bivalents, the hexaploid complement. Cytomyxis is found in this stage and in the preceding spireme condition of the mother cells. Instances of non-paired chromosome mates may be seen on the heterotypic spindle. For the most part the divisions are quite normal, however, and consequently no polycary is found.

Echinochloa frumentacea (Roxb.) Link; introduced from the Orient as an escape from cultivation. HITCHCOCK (22) terms this species *Echinochloa crus-galli* (L.) Beauv. var. *edulis* Hitchc.; pollen 30–40 per cent imperfect.—Counts of metaphase plates of the heterotypic division show the presence of 28 bivalents, the octoploid number. Examples of loose pairing and lagging may frequently be found in the heterotypic metaphase, but only occasionally do bivalents lag in the anaphase. Homeotypic divisions and tetrads are quite normal in appearance.

ANDROPOGONEAE

Miscanthus sinensis Anderss. var. *zebrinus* Beal, cultivated for ornament; pollen 40 per cent imperfect.—Heterotypic metaphase plates clearly show 21 bivalent chromosomes. Cytomyxis, although a common phenomenon at the spireme stage, is present to a remarkable degree in this species. Fully two-thirds of the spireme, including the nucleolus, may migrate to an adjacent mother cell. It is not uncommon to see the nucleoli of two cells fused in the midst of a migrating mass of chromatin. The phenomenon often persists through diakinesis and the beginning of the heterotypic division. An occasional case is found where the heterotypic spindle is initiated between two mother cells, obviously the result of cytomyxis in earlier stages. Occasional laggards may be observed in the first division, the mother cells often displaying large and numerous connections of cytoplasm with neighboring cells. It is not uncommon to find chromatin extruded in the telophase. Cytomyxis may be observed again in the telophase as well as at interkinesis. Lagging with accompanying extrusions is present to a similar degree in the homeotypic divisions. Polycary is rarely found, however, and is not very striking when it does occur.

Andropogon scoparius Michx.; pollen 20–30 per cent imperfect.—This species is octoploid, having the unusual complement of 21 bivalents and 14 univalents. The bivalents are easily counted in the diakinesis and at the heterotypic metaphase plate (fig. 77). The best estimation of the number of univalents is obtained in the spindle view of the metaphase of the first division (fig. 76), during which they are seen lagging in contrast to the bivalents at the plate. The size of the univalents is quite striking, being not more than half that of the univalents that undergo pairing. Many of these univalents never reach the plate, as is evidenced in the partly completed anaphase (fig. 78). Those univalents that reach the middle of the spindle are distributed at random to the poles, not dividing in the process. The diad stage may be observed with extrusions, chromatin at times being stranded in the space between the separating halves of the mother cell. Bivalents may occasionally lag with the univalents in the homeotypic division (fig. 79), the latter pursuing the same course as in the first division. Polycary is quite common in the tetrads (fig. 80).

Andropogon furcatus Muhl.; pollen 10–20 per cent imperfect.—This species is decaploid, heterotypic metaphase plates showing 35 bivalents (fig. 83). Diakinesis displays a striking ring formation of the synaptic mates. Barring a sluggishness of chromosome action in the early stages of both metaphase and anaphase, the heterotypic division is quite regular (figs. 82, 84). Similar regularity is observed in the homeotypic division (fig. 85), and the tetrads have a normal appearance (fig. 86).

Sorghastrum nutans (L.) Nash; pollen 10–20 per cent imperfect.—Diakinesis and heterotypic metaphase plates clearly show 20 bivalents, the tetraploid number. Occasionally a laggard may be seen in the metaphase of the first division, but regularity is the rule. Not only does the bivalent nature of the chromosomes reveal itself at this stage, but often the homeotypic split is manifested in an incipient condition, the whole presenting a linear tetrad appearance. Anaphases frequently display considerable irregularity in the early stage, but the late condition is quite normal. Cytomyxis, present in the prophase, may be observed occasionally at interkinesis. The homeotypic division is regular in appearance, as are the tetrads.

Discussion

POLYPLOIDY

In the preceding article, the phenomenon of polyploidy was discussed as an important criterion of hybridization. Of special interest in the present reported series are the hexaploids *Panicum dichotomiflorum*, *Echinochloa crus-galli*, and *Miscanthus sinensis* var. *zebrinus;* the octoploids *Echinochloa frumentacea* and *Andropogon scoparius;* and the decaploid *Andropogon furcatus.*

DYSPLOIDY

A further numerical comparison of the chromosome complements of the species of grasses investigated reveals some instances where the haploid number is not a definite multiple of the basic number for the group. For example, in *Phalaris* the usual series of 7 holds for *P. arundinacea* and its tetraploid variety *picta;* yet *P. canariensis* has a haploid number of only 6. Again, in *Panicum* a new series of 9 appears to be established Irregular deviations from even this factor are found, however In *P. lindheimeri* var. *typicum* and in the variety *fasciculatum*, counts of 8 instead of 9 are frequently obtained. In *P. subvillosum*, not only counts of 8, but counts of 7 and 10 may be instanced. Although *P. dichotomiflorum* seems to hold to the 9 series with 27 bivalents, *P. miliaceum* only occasionally shows 18 bivalents, 20 being the more established haploid complement. Table I shows that 10 is a well established number in *Paspalum*, yet counts of 9 and of 11 may be obtained in *P muhlenbergii*. Such irregular deviations from the fundamental haploid base in a polyploid series have been conveniently termed dysploids (JEFFREY 30).

Previous investigation in various genera of grasses has displayed similar cases of dysploidy. The new haploid number of 12 has been reported in *Oryza sativa* (ISHIKAWA 27), yet counts of 10 to 14 have been made in several so-called "race mutants" of this species (NAKATOMI in AASE and POWERS 1). Races of *Hordeum* (KIHARA 32) and *Secale* (GOTOH 19) with 8 as the haploid number instead of the usual 7 have been reported. Deviations from the haploid count of 10 are found in *Euchlaena mexicana* (LONGLEY 35) and certain races of *Zea mays* (KUWADA 34). Segregates of *Avena sativa* show 40, 41, or 44 instead of the usual somatic complement of 42 (HUSKINS 26). A considerable amount of dysploidy is seen in various species and hybrids in *Saccharum* (BREMER 3, 4). The obvious hybrid origin of many of these dysploids is particularly significant.

Outside of the Gramineae, hybridity and dysploidy may again be frequently correlated. *Oenothera* "mutants" are found with 15 and 16 chromosomes instead of the usual 14 (LUTZ 36). Very irregular series are found in the much inter-crossed species of *Crepis* (ROSENBERG 41) and *Lactuca* (ISHIKAWA 28).

ABSENCE OF POLYPLOIDY.—Before leaving the general subject of polyploidy and its relation to hybridization, the converse situation must be considered; namely, the absence of chromosome multiplication where other obvious evidence of crossing is present. It has been noted that in the 8 forms of the *dichotomum*-like species of *Panicum* studied there is no multiple deviation from the haploid number of 9; yet these forms bear just as obviously the characteristics of hybrids

TABLE I

EXTENT OF POLYPLOIDY IN THE GRAMINEAE

	DIPLOID	TETRAPLOID	HEXAPLOID	OCTOPLOID	DECAPLOID
Digitaria					
sanguinalis*		14			
Paspalum					
stoloniferum (Marchal 37)	10				
dilatum (Marchal 37)		20			
muhlenbergii*	10				
Panicum					
dichotomiflorum*			27		
miliaceum*		20			
lindheimeri* (4 vars)	9				
subvillosum*	9				
tsugetorum*	9				
sphaerocarpon*	9				
scribnerianum*	9				
Echinochloa					
crus-galli*			21		
frumentacea*				28	
Imperata					
arundinacea (Bremer 4)	10				
Miscanthus					
sinensis v zebrinus*			21		
Ischaemum					
timorense (Bremer 4)	10				
Saccharum (Bremer 4)			30	40	
Erianthus (Bremer 4)			30		
Andropogon					
scoparius*				21 bivalents +14 univalents	
furcatus*					35
Cymbopogon (Kuwada 33)	10				
Sorghum (cf Ishikawa 27)	10				
Sorghastrum					
nutans*		20			
Tripsacum (Longley 35)			30		
Euchlaena (Longley 35)	10	20			
Coix (Longley 35)	10				
Zea (cf Gaiser 13)	10				

* Species investigated in this research Numbers refer to the haploid complement

in their closely intergrading external characters, sterile pollen, polycary, and lagging chromosomes.

While perhaps a satisfactory explanation of such a case is lacking, it is not without parallel instances. The most striking case among the Gramineae is that of the 37 varieties of *Hordeum vulgare* all showing a haploid number of 7 (EMME 9). The same count holds for the 10 other species of the genus investigated (GAISER 13). Many of the species and varieties of *Aesculus* are bad hybrids (HOAR 24), yet, except in one instance, they are all diploids. Similar situations may be cited in *Wisteria* (ROSCOE 38) and *Anthurium* (GAISER 14).

LAGGING UNIVALENTS.—In the previous article, the presence of 14 univalents in the hexaploid *Spartina alterniflora* var. *glabra* was compared with similar conditions in several well known natural and experimental hybrids.

The presence of univalents in octoploid types of a regular polyploid series, such as is found in *Andropogon scoparius* (with 21 bivalents and 14 univalents) is apparently rare. Analogies appear, however, in connection with high multiples in dysploid series of hybrids. Among these are the varieties of *Saccharum officinarum*. The type species shows regularly 40 bivalents, but in "Black Cheribon" for example, 36 bivalents and 8 univalents appear (BREMER 3). High chromosome numbers involving lagging univalents are seen again in sterile fern hybrids, as *Polypodium schneideri* (FARMER and DIGBY 10) and *Nephrolepis exaltata* var. *bostoniensis* (JEFFREY and HICKS 31).

NON-PAIRING, LAGGING AND EXTRUSION.—In the Festuceae, instances have been noted where unpaired chromosome mates in diakinesis are seen to lag on the spindle of the first division, and later become extruded into the cytoplasm. *Echinochloa crus-galli* and *E. frumentacea* both display similar laggards on the heterotypic spindle. In *Miscanthus sinensis* var. *zebrinus* such univalents never reach the plate during the first division, but remain at the poles. *Panicum lindheimeri* var. *typicum* and *P. sphaerocarpon* both show a considerable amount of non-pairing at diakinesis. Again, it was noted in *P. subvillosum*, *P. tsugetorum*, and *P. scribnerianum* that loosely associated bivalents often lag in the heterotypic metaphase. These conditions, together with those of lagging and extrusion of bivalents in both maturation divisions, such as are found in *Paspalum muhlenbergii* and several species of *Panicum*, have been correlated previously with the same conditions in known or suspected hybrids.

NON-DISJUNCTION.—In the case of *Panicum lindheimeri* var. *fasciculatum*, a definite case of non-disjunction has been demonstrated in the heterotypic division (figs. 59, 60). This is the particular type of unequal chromosome distribution in which one or two bivalents may approach and nearly reach the equatorial plate, but are pulled undivided to one of the poles. Instead of the usual 9/9 segregation in the anaphase, counts of 10/8 or 11/7 (fig. 61) are consequently made, with a resultant difference in the chromosome complements of the pollen grains if they mature.

Non-disjunction has been reported frequently in hybrids. *Oenothera lamarkiana* may give anaphase segregations of 8/6 or 9/5 instead of the usual 7/7 (SINOTO 44). In the tetraploid species of *Datura*, segregates of 23/25 instead of 24/24 are quite regularly found (BELLING and BLAKESLEE 2). The same situation has been recently reported in *Nicotiana alata* var. *grandiflora*, where 8/10 may often be the count in the anaphase instead of 9/9 (RUTTLE 42).

When gametes of different numerical chromosome equipments mature as a result of unequal distribution or non-disjunction, it is obvious that they are functional in producing dysploid offspring, the many instances of which have been cited in a discussion of that particular type of polyploidy. Such is the opinion of HEILBORN (21) regarding the striking dysploid series in *Carex*. SHARP (43) states:

> Nothing is more reassuring in cytology at the present time than what we are able to ascertain concerning the rôle of chromosomes from their occasional misbehavior. In such phenomena as non-disjunction and polyploidy, nature performs almost before our eyes a series of experiments from which we should be dull indeed if we were to learn nothing......... We have almost everything to learn about the causes of such aberrations, but it is already clear that they play a part in the production of new races; and when we consider the multiploid series of species in many genera and families we can have little doubt that they have functioned in the origin of species also.

POLYSPORY.—In an investigation of 31 species and varieties of grasses, sterile pollen has been correlated repeatedly with polycary. It is noteworthy, however, that a representative from the much hybridized group of *Panicum*, *P. lindheimeri* var. *typicum*, presents apparently the only case of polyspory in the Gramineae yet reported.

CYTOMYXIS.—The phenomenon of cytomyxis has been noted often in the cytological descriptions of the grasses in this study. While the pollen mother cells are in the spireme condition, considerable quantities of chromatin may be ejected from the nucleus and incorporated into the cytoplasm of adjacent mother cells. Anthers displaying more advanced stages show many mother cells with cytoplasmic chromatin, and adjacent shrunken remains of cells that have given up the greater portion of their nuclear contents, clearly demonstrating the result of excessive ejection of spireme chromatin. This type of chromatin loss has been termed cytomyxis (GATES 16).

During the course of spireme cytomyxis, cytoplasmic strands may be formed in varying degrees of number and size between the mother cells. These intercommunicating strands often persist until the homeotypic division is well under way. They usually disappear, however, by the time the tetrads have formed. Chromatin or chromosomes stranded between cells in diakinesis, heterotypic metaphase, or interkinesis may or may not be assisted in migration by cytoplasmic bridges.

The first type of the grasses displaying cytomyxis are those in which the phenomenon is seen only in the spireme stage, but with resultant extrusions persisting in later stages. These are *Andropogon scoparius*, *Digitaria sanguinalis*, *Spartina michauxiana*, and *Festuca rubra*.

A greater number of the species investigated show such chromatin loss to the extent that the process is still manifested in diakinesis. Among these have been noted *Ammophila breviligulata*, *Alopecurus pratensis*, *Phalaris canariensis*, *Paspalum muhlenbergii*, and *Echinochloa crus-galli*. Cytomyxis has been observed in abundance at diakinesis in the dichotomum type of *Panicum*. Whereas all of these species are very sterile, it is interesting to note that the greatest amount of cytomyxis is correlated with the greatest amount of irregularities in the maturation divisions. A series may thus be arranged to show: a first group of *P. lindheimeri* var. *typicum*, *P. subvillosum*, and *P. scribnerianum;* a second group of *P. lindheimeri* var. *fasciculatum* and *P. tsugetorum;* and a third group of *P. lindheimeri* var. *implicatum* and *P. sphaerocarpon.*

An excessive amount of cytomyxis in all stages of the prophase may result in irregularities in the heterotypic metaphase in which chromosomes on or off the spindle may be partly stranded in adjacent cells. This condition has been noted in *Alopecurus geniculatus* var. *aristulatus* (occasionally in *A. pratensis*), *Spartina alterniflora* var. *glabra*, and *Phalaris arundinacea* var. *picta*. In the case of *Miscanthus sinensis* var. *zebrinus*, the heterotypic spindle has been seen stranded between two mother cells.

Similar phenomena of chromatin transference at times are observed during interkinesis. Among such cases are *Miscanthus*, where cytomyxis has been observed in all stages, and the following where it has been observed previously in prophase: *Festuca duriuscula*, *Panicum dichotomiflorum*, *P. miliaceum*, and *Sorghastrum nutans.*

Cytomyxis has often been reported in pollen mother cells, although naturally in the light of various interpretations. GREGORY (20) noted the exchange of spireme chromatin in a sterile race of *Lathyrus odoratus*, and thought it to be some peculiar type of cell division. ROSENBERG (40) noted spireme cytomyxis in *Drosera longifolia* and SINOTO (45) in *Iris japonica*. Both of these investigators believe the phenomenon to be an artifact due to faulty fixation.

DIGBY (5) noted the ejection of chromatin during the spireme stage in *Galtonia candicans*. Inasmuch as nucleolar chromatin is believed to be involved, the ejections are termed nucleolar buds. They do not persist after the prophase. Figures published later (DIGBY 6) concerning the reduction division of this *Galtonia* seem to indicate obvious hybrid characteristics. "Chromatic" or "nucleolar droplets" of the same origin have been reported in *Polypodium schneideri* (FARMER and DIGBY 10), *Primula kewensis* (DIGBY 7), and *Crepis virens* (DIGBY 8), concomitant hybrid divisions being noted in all instances. That cytomyxis can be responsible for the degeneration of pollen mother cells and even whole anther sacs has been recognized, although strangely enough the investigators cite such conditions as normal. This is the opinion of FRASER (12) reporting on *Vicia faba*, and of WEST and LECHMERE (47) on *Lilium candidum.*

Cytomyxis recognized as an abnormality has been reported in several definitely known hybrids. At the spireme stage it has been seen in *Oenothera rubrinervis* (GATES 15) and *O. gigas* (GATES 16). A hybrid form of *Typha angustifolia* shows the phenomenon at diakinesis (ROSCOE 39). Instances of its further appearance at interkinesis are found in *Lactuca sativa* (GATES and REES 18) and *Lathrea squamaria* (GATES 17).

It will be noted in a comparison between the grasses in this research and the cases of cytomyxis just cited, that the present study reveals a wider extent of the abnormality throughout the stages of the maturation divisions. Cytomyxis at the spireme stage is rather common, but it has been reported but once in diakinesis and apparently not at all in the heterotypic division such as is described in *Alopecurus*, *Phalaris*, and *Spartina*. The case of *Miscanthus* showing a spindle stranded between two mother cells seems to be thus far unique; yet, in all of these cases the cytoplasm of the cells involved shows absolutely no shrinkage. An occasional instance of an anther that has not been infiltrated with nitrocellulose, due to a failure in pricking, displays much distorted cytoplasm in the mother cells but scarcely any dislodgment of the chromatin, and certainly no ejection into neighboring cells, particularly if the anther in question happens to have reached the diakinesis or a later stage. Furthermore, the employment of such a rapid fixative as Carnoy's fluid together with an exhaust pump has not produced shrinkage in anthers displaying more normal conditions in the same material in question; hence it would seem that the phenomenon of cytomyxis cannot be an artifact. On the other hand, the occurrence of this abnormality in connection with hybrids is very striking. In contrast, the examination of considerable material in the case of *Phalaris arundinacea*, a normal diploid, has revealed no cytomyxis. Finally, even if the phenomenon can be proved not to be an exclusively hybrid characteristic, its occurrence is manifestly increased in obvious hybrids such as are described in *Panicum*.

Conclusions

PANICEAE

Digitaria sanguinalis.—This very common weed is representative of a widespread genus of some 60 species, 12 occurring in the southeastern United States. The species here considered is listed as being very variable in GRAY's manual, yet so far as the material investigated reveals, this is another "settled down" tetraploid species like *Dactylis glomerata* and *Spartina michauxiana*. In these forms the outer evidence points to hybridity, yet cytologically such evidence is concealed. Except for the presence of some bad pollen, these cases suggest those termed crypthybrids by JEFFREY (29).

Paspalum muhlenbergii.—This genus has about the distribution of *Digitaria*, with some 200 species, 50 of which are in the United States. The one here considered shows hybrid tendencies in lagging chromosomes, sterile pollen, and frequent irregularities in chromosome count.

Panicum.—This huge widespread genus of 500 species, with about 150 in the United States, presents a promising group for the appearance of hybrids, as is shown in the following subgenera:

Dichotomiflora.—The hexaploid *P. dichotomiflorum*, displaying irregularities in the reduction division and sterile pollen, must have a hybrid ancestry. It is noteworthy that transitional varieties to the next section have been found (SVENSON 46).

Capillaria.—*P. miliaceum* seems to be a fairly settled species, yet occasional lagging chromosomes, bad pollen, and differences in the chromosome count indicate an uncertain purity of origin.

Dichanthelium.—Representatives of four tribes in this subgenus all show evidence pointing to a considerable degree of hybridization between the species. Although polyploidy is absent, there is presented extreme variability, very sterile pollen, much lagging in both maturation divisions, irregularities in chromosome count, cytomyxis, polycary, and even polyspory in one instance, the last phenomenon being apparently rare in the Gramineae.

Echinochloa.—This is not a particularly large genus, there being known only 10 species, with 4 in the United States. A rather high degree of polyploidy is reached, with the hexaploid number in *E. crus-galli* and the octoploid in *E. frumentacea*.

ANDROPOGONEAE

Miscanthus sinensis var. *zebrinus*.—The apparent hexaploid count of 21 bivalents seems to be anomalous in this species, since it deviates from the usual 10 series of related genera, particularly *Sac-*

charum. Deviations are not unknown, however, even in this latter genus (GAISER 13). Lagging chromosomes, much cytomyxis, as well as sterile pollen point to an evident hybrid ancestry for this variety. BREMER (4) has investigated several species in closely related genera of this tropical group, and considers many of them hybrids on the basis of polyploidy alone.

Andropogon.—Here again we are dealing with a large tropical genus of about 150 species, some 30 of which are found mostly in the southeastern United States. Certainly if polyploidy, even of itself, is a criterion of hybridity, it is here very strikingly instanced in the octoploid *A. scoparius* and the decaploid *A. furcatus. A. scoparius* has been noted in several varieties in this region (HUBBARD 25), and the presence of univalent chromosomes, polycary, and sterile pollen makes the evidence overwhelming for its hybrid origin. *A. furcatus* is equally outstanding in its regularity of reduction divisions and only very small amount of sterile pollen. Its hybrid origin may be more remote than that of *A. scoparius*, but its decaploidy attests such a lineage as has been previously stated.

Sorghastrum nutans.—This species is representative of a genus of 10 species, 3 of which occur in the United States, east of the Rockies. The evidences for hybridization as a factor in its origin (tetraploidy and a slight amount of chromosome irregularity and pollen sterility) are not striking, but point to the possibility nevertheless.

Summary

1. The following representatives of the tribes Paniceae and Andropogoneae have been investigated:

DIPLOIDS.—*Paspalum muhlenbergii, Panicum lindheimeri* in its four varieties, *P. subvillosum, P. tsugetorum, P. sphaerocarpon, P. scribnerianum.*

TETRAPLOIDS.—*Digitaria sanguinalis, Panicum miliaceum, Sorghastrum nutans.*

HEXAPLOIDS.—*Panicum dichotomiflorum, Echinochloa crus-galli, Miscanthus sinensis* var. *zebrinus.*

OCTOPLOIDS.—*Echinochloa frumentacea, Andropogon scoparius.*

DECAPLOID.—*Andropogon furcatus.*

2. Lagging univalents are found in *Andropogon scoparius.*

3. Non-disjunction is reported in *Panicum lindheimeri* var. *fasciculatum.*

4. Polyspory is found only in *Panicum lindheimeri* var. *typicum.*

5. Irregularities in the chromosome count are reported in several members of *Panicum.*

6. Cytomyxis is found to extend throughout the diakinesis and heterotypic metaphase in several species.

7. Varying degrees and combinations of non-pairing, lagging and extrusion, cytomyxis, polycary, and sterile pollen are found in the species investigated.

8. Polyploidy or cytological abnormalities of the maturation divisions or both are considered as evidence of the hybrid origin of these species.

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EXPLANATION OF PLATES IV–VI

PLATE IV

× 2540 diameters

Panicum lindheimeri Nash var. *typicum* Fern.

FIG. 44.—Diakinesis showing 9 bivalents, one loosely paired; note cytoplasmic chromatin.

FIG. 45.—Early heterotypic anaphase, some bivalents late in splitting.

FIG. 46.—Heterotypic anaphase.

FIG. 47.—Heterotypic telophase; chromatin in path of obliterated spindle.

FIG. 48.—Somatic division (cell from young ovule tissue) contrasting with reduction division.

FIG. 49.—Heterotypic metaphase, polar view, showing 9 bivalents.

FIG. 50.—Homeotypic metaphase showing laggards.

FIG. 51.—Homeotypic anaphase showing laggards.

FIG. 52.—Homeotypic metaphase, polar view, revealing only 8 chromosomes.

FIG. 53.—Homeotypic anaphase, irregular division.

FIG. 54.—Homeotypic anaphase.

FIG. 55 —Tetrad showing polycary and polyspory.

FIG. 56 —Abortive pollen grain.

Panicum lindheimeri Nash var. *fasciculatum* (Torr.) Fern.

FIG. 57.—Heterotypic metaphase showing laggards.

FIG. 58.—Heterotypic anaphase showing laggards.

FIG. 59.—Heterotypic anaphase; note collapsed spindle.

FIG. 60.—Same; note non-disjoined bivalents passing to one pole.

FIG. 61.—Heterotypic telophase; note different number of chromosomes in nuclei.

PLATE V

×2540 diameters

Panicum sphaerocarpon Ell.

FIG. 62.—Diakinesis showing 9 bivalents, several loosely paired.

FIG. 63.—Heterotypic metaphase; note laggards.

FIG. 64.—Heterotypic anaphase.

FIG. 65.—Heterotypic telophase; note chromatin extruded from nuclei and cytomyxis.

FIG. 66.—Homeotypic metaphase; note extrusions and laggards.

FIG. 67.—Two polar views of homeotypic metaphases showing 8 and 10 counts.

FIG. 68.—Homeotypic anaphase.

Panicum scribnerianum Nash

FIG. 69.—Diakinesis in 3 mother cells showing cytomyxis.

FIG. 70.—Diakinesis in 2 mother cells, lower having nearly 2 chromosome sets as result of cytomyxis.

FIG. 71.—Early heterotypic metaphase; note extrusions.

FIG. 72.—Heterotypic anaphase; note bivalent laggards.

FIG. 73.—Homeotypic metaphase; note laggards and extrusions.

FIG. 74.—Homeotypic telophase; note extrusions.

FIG. 75.—Tetrad showing polycary.

PLATE VI

×2300 diameters

Andropogon scoparius Michx.

FIG. 76.—Heterotypic metaphase showing bivalents at plate and 14 univalent laggards on spindle.

FIG. 77.—Heterotypic metaphase, polar view, showing 21 bivalents and 12 univalents.

FIG. 78.—Heterotypic anaphase; some univalents still at poles.

FIG. 79.—Homeotypic divisions; both bivalent and univalent laggards in metaphase.

FIG. 80.—Tetrad showing polycary.

FIG. 81.—Somatic anaphase (cell from young ovule tissue); note regularity.

Andropogon furcatus Muhl.

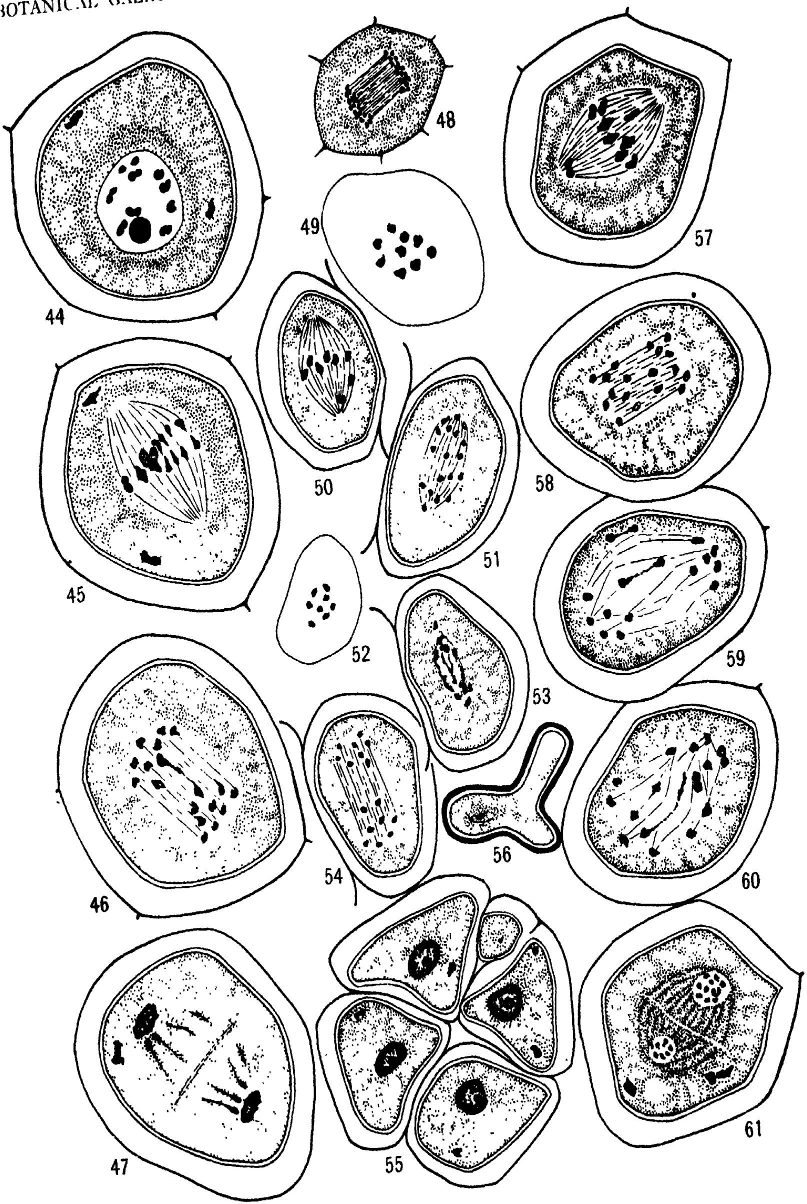
FIG. 82.—Heterotypic metaphase.

FIG. 83.—Heterotypic metaphase, polar view, showing 35 bivalents.

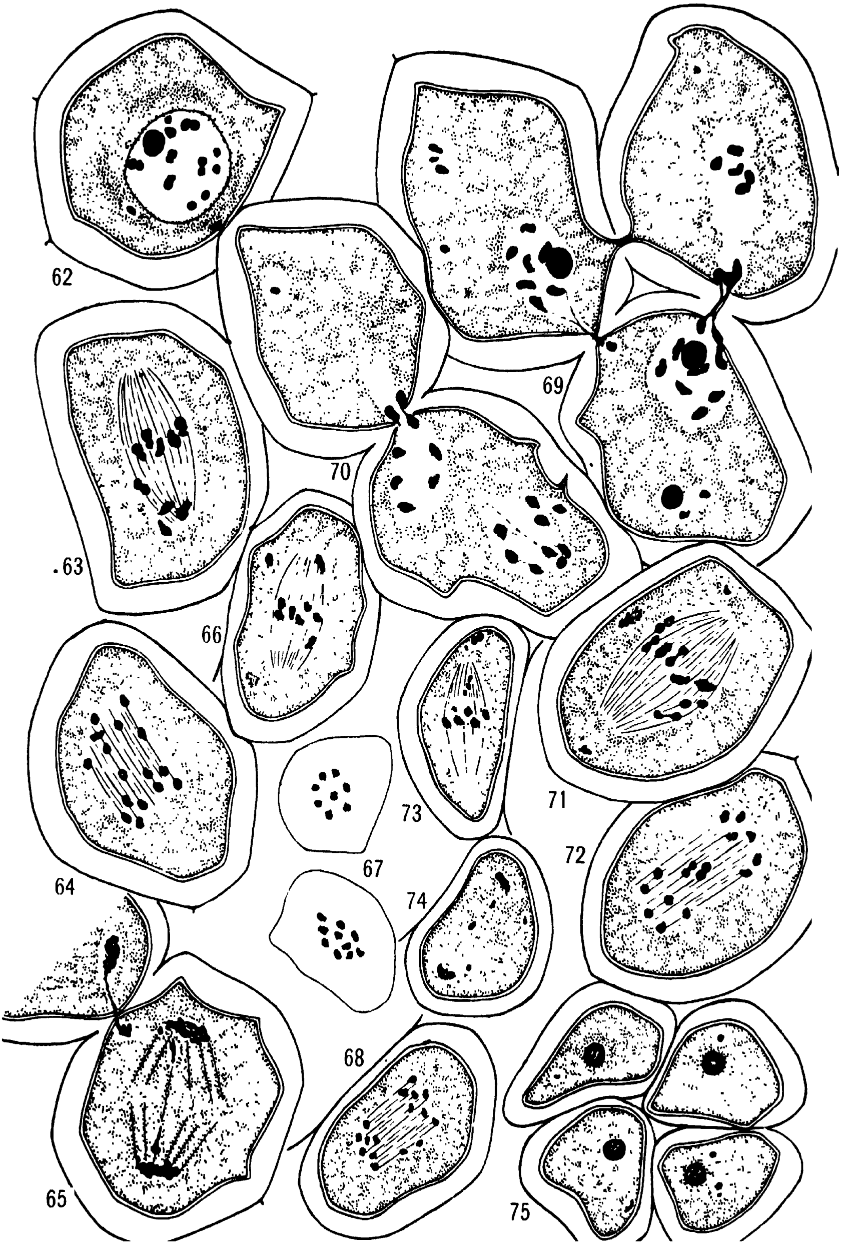
FIG. 84.—Heterotypic anaphase.

FIG. 85.—Homeotypic divisions.

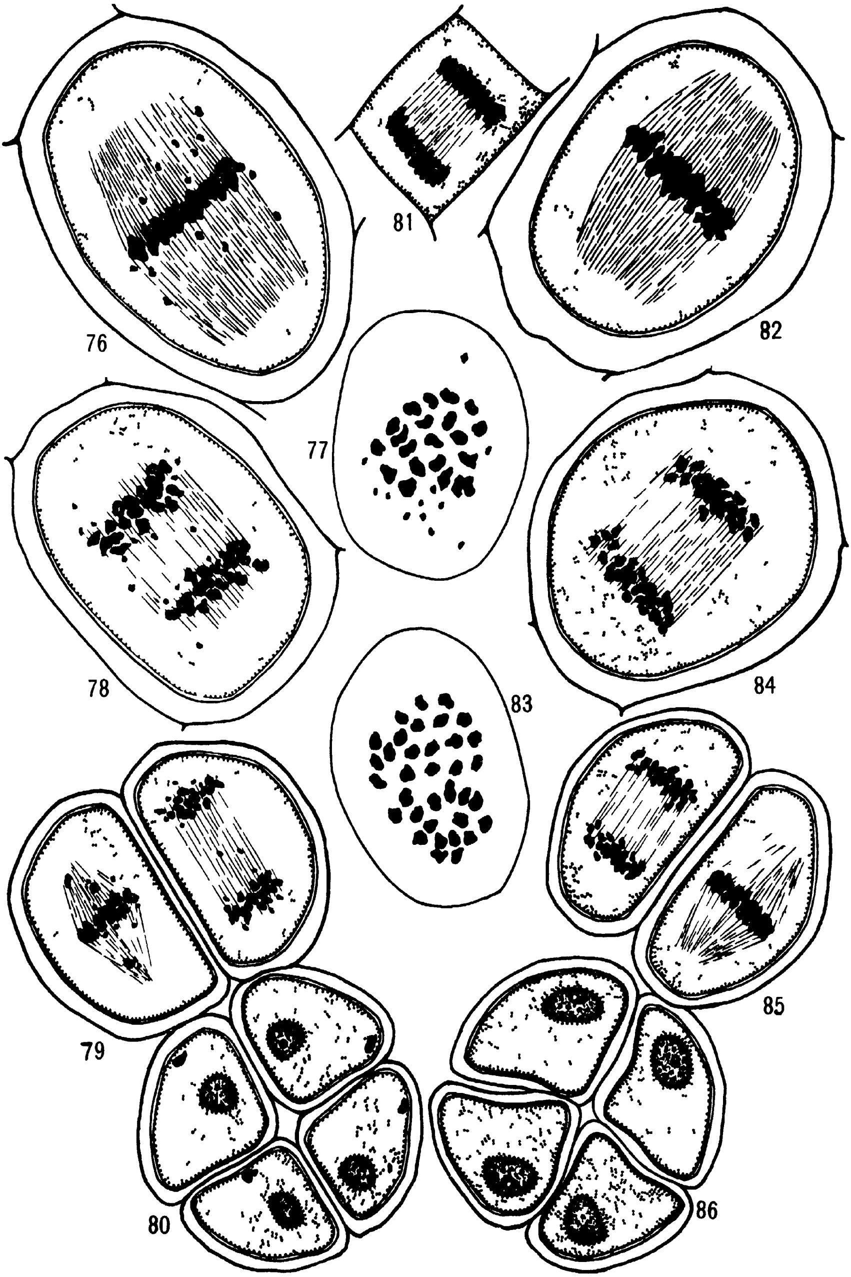
FIG. 86.—Tetrad presenting normal condition in contrast to fig. 80.



CHURCH on GRAMINEAE



CHURCH on GRAMINEAE



CHURCH on GRAMINEAE

EFFECT OF MINERAL NUTRIENTS UPON SEED PLANTS

II. PHOSPHATES[1]

THOMAS W. TURNER

Introduction

That phosphates stimulate root growth in plants is generally accepted in agricultural practice, as well as in the plant nutrition laboratory; yet the experimental evidence in support of this statement is surprisingly little. A review of the literature of the present day concerning the effects of phosphates upon plant growth shows but slight advance over the information reported from Rothamsted by LAWES and GILBERT (1) in 1847. More recent experiments reported from the same station (4, 5), and others carried on both in the field and in the laboratory at various agricultural experiment stations (2) in this country, confirm the main points of the early conclusions. SHULL (6) has brought together the main points of the phosphate problem as follows:

> In summarizing the effects of phosphates on plant growth, RUSSELL calls attention to increased growth of root system in lateral and fibrous roots and the better storage of food in root crops provided with an abundance of suitable phosphates. He mentions the influence of this element in increasing both straw and grain production, its connection with nuclear processes in cell division, its relation to normal transformations of starch, and its importance in determining the quality and feeding value of crop grown in the soil.

It might be added that investigations reported so far show that the effects of phosphates differ with the kinds of plants used, as well as with the external factors surrounding them as they grow. If we regard the general statement as demonstrated that these salts bring about an increased development of roots, we still must show whether or not this effect is due to the salt acting directly upon or within the roots, or whether it may be due to some retardation in the use of the food by the aerial part which leaves it for translocation and storage or other utilization in the roots. This paper reports some results of experiments undertaken to determine whether or not the salt effects are brought about by direct effect upon the root.

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[1] Contribution from Biological Laboratory of Hampton Institute. These experiments are a part of a series begun in the Laboratory of Physiology of Cornell University, at the suggestion of Professor O. F. CURTIS, and carried on now for the past three years in the Biology Laboratory of Hampton Institute.

Material and methods

The same general methods of water culture experimentation have been followed in this investigation as was used in earlier studies reported in detail elsewhere (8). An interesting feature of this method is that it conveniently makes use of nine salts, as follows:

$Ca(NO_3)_2$	KNO_3	$Mg(NO_3)_2$
$CaHPO_4$	KH_2PO_4	$Mg(PO_4)_2$
$CaSO_4$	K_2SO_4	$MgSO_4$

The use of such an arrangement of the nutrient salts makes the omission of cations or anions a simple matter, and does not necessitate introducing other so-called non-nutrient elements, which is the usual case. On the other hand, the total concentration of the solution (including the concentration of cations or anions) is lowered by this method, but preliminary studies showed that the concentrations employed had no harmful effects upon growth of the plants used. In the two solutions used (P_1, low phosphate and P_2, high phosphate), the quantity of phosphates in the high was twenty times that in the low, the other nutrients being the same in both. The salts in solution were in the following proportions:

	LOW PHOSPHATE SOLUTION (PER LITER)	HIGH PHOSPHATE SOLUTION (PER LITER)
$Ca(NO_3)_2$	0.240	0.240
$Mg(NO_3)_2 \cdot 6H_2O$	0.287	0.287
KNO_3	0.228	0.228
$CaSO_4$	0.179	0.179
$MgSO_4$	0.167	0.167
K_2SO_4	0.136	0.136
$Ca(HPO_4) \cdot 2H_2O$	0.021	0.420
$Mg_3(PO_4)_2 \cdot 4H_2O$	0.014	0.280
KH_2PO_4	0.0167	0.334
$FeSO_4$	0.033	0.033

The seeds were sterilized with formalin 1 part to 500 for 20 minutes, thoroughly washed with running tap water, and germinated between blotting paper on earthen plates. The seedlings were grown in quart jars, four to a jar.

Distilled water was used for all culture solutions, and Baker's analyzed chemicals were used throughout the experiment. The solutions were changed once a week, while the initial hydrogen concentration of the solutions as prepared ranged from pH 5.8 to 6.3. Care was taken to have the pH values the same for the low and high phosphate solutions at the beginning of each series and at each change of solution. The colorimetric method was used for determining the pH values, and the adjustments of the same were made with N/20 NaOH or 0.1 per cent HCl when it was necessary to bring the solutions to the same pH. The osmotic pressures of the two solutions used were not determined, but there was no indication of any injury in growth due to the greater concentration of the high phosphate solution.

The method of getting quantitative data was the same as that reported in a former paper (8). In brief, while other salt quantities than phosphates were kept approximately constant, the ratios of growth of tops to growth of roots for particular periods were determined by weight. Any marked variation in the ratio of tops to roots under the conditions would be attributed to the varying quantities of phosphates in the two solutions. The second question to be determined, namely, whether or not this variation was the result of an accelerating effect of the salt acting directly upon the roots, was approached through the employment of Robbin's method of growing root tips under sterile conditions in the solutions already described. Dextrose to the amount of 1 per cent was added to the solutions to supply the necessary carbohydrates. Barley, wheat, and cotton were the plants used, but the root tips of corn only were tried.

Results

Experiment 1: barley

This experiment extended over 23 days and was divided into two series, one covering a 16-day period, the other extending over the entire 23 days. In all cases the cultures were set up at the same time and were grown under the same greenhouse conditions. Four jars, making a total of sixteen plants, were harvested and weighed at each series. Table I gives the results of the first set of experiments dealing with barley seedlings.

TABLE I

EFFECT OF INCREASING PHOSPHATE CONCENTRATION ON RATIO OF TOP TO ROOT GROWTH IN BARLEY SEEDLINGS (GREEN AND DRY WEIGHTS IN GRAMS)

		SERIES I (16 DAYS)			SERIES II (23 DAYS)		
		Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Solution 1, low phosphate...	Green weight	4.3204	0.9313	4.64	5.5533	1.0668	5.21
		4.318	0.8358	5.17	4.9135	0.9405	5.22
		3.4212	0.7732	4.42	5.7146	1.0713	5.33
		3.5964	0.7860	4.58	4.9290	0.9100	5.42
	Total....	15.6560	3.3263		21.1104	3.9886	
	Mean....	....	....	4.70			5.30
	Dry weight	0.3446	0.0495	6.96	0.4641	0.0605	7.67
		0.3318	0.0528	6.28	0.4435	0.0630	7.04
		0.2790	0.0463	6.03	0.5000	0.0670	7.46
		0.2664	0.0482	5.53	0.4368	0.0558	7.83
	Total.....	1.2218	0.1968		1.8444	0.2623	
	Mean....	......	..	6.20			7.50
Solution 2, high phosphate...	Green weight	3.5263	0.9070	3.89	4.2060	0.9824	4.28
		3.0346	0.6814	4.45	4.2537	0.8671	4.91
		2.6900	0.7339	3.67	5.8868	1.0410	5.65
		3.6600	0.7158	5.11	3.6552	0.9544	3.83
	Total....	12.9109	3.0381		18.0017	3.8449	
	Mean....	....	....	4.28			4.67
	Dry weight	0.2906	0.0583	4.98	0.3797	0.0758	5.01
		0.2440	0.0426	5.73	0.3707	0.0655	5.66
		0.2324	0.0518	4.49	0.4839	0.0763	6.34
		0.2922	0.0543	5.38	0.3200	0.0690	4.64
	Total.....	1.0592	0.2070		1.5543	0.2866	
	Mean.....	....	....	5.15			5.41

In series I (16-day period), the mean of the ratios of top to root growth for the four cultures was 6.20 for the dry weight in the low phosphate solution, and 5.15 for those in the high phosphate. The difference between them is 1.05. The green weights showed ratios in the same order, although not quite so marked, being 4.70 for low and 4.28 for the high phosphate. In series II (23-day period) the mean ratios for the dry weights were 7.50 for the low phosphate and 5.41 for the high phosphate, the difference being 2.09.

Growth characters.—The high phosphate cultures were more rigid and the leaves more erect, otherwise they appeared equally green and healthy. No experiments have been attempted so far to show whether or not the differences in rigidity and erectness of these plants may be explained on an anatomical basis or on the basis of a difference in concentration of the solution surrounding the cells. It will be seen as one compares the difference in these ratios that there is a 100 per cent increase in case of the 23-day period over the 16-day for the dry weights. The higher phosphate concentration makes for decreasing ratio of tops to root. Nitrates were demonstrated to have the opposite effect in barley (**8**).

TABLE II

EFFECT OF INCREASING PHOSPHATE CONCENTRATION ON RATIO OF TOP TO ROOT GROWTH IN WHEAT SEEDLINGS (GREEN AND DRY WEIGHT IN GRAMS)

		SERIES I (15 DAYS)			SERIES II (22 DAYS)			SERIES III (29 DAYS)		
		Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Solution 1, low phosphate....	Green weight	2.6586	1.1420	2.33	5.7944	1.3634	4.25	5.7435	1.1400	5.04
		3.1630	1.3442	2.35	4.4580	1.2834	3.47	5.5158	1.1738	4.7
		3.2848	1.4286	2.3	4.4528	1.3102	3.39	6.5370	1.9618	6.1
		2.744	1.2746	2.15	5.7403	1.5470	3.71	5.8019	1.5153	4.3
	Total.....	11.8308	5.1794		20.4455	5.5040		23.5982	4.7909	
	Mean.....	....	....	2.3			3.7			5.03
	Dry weight	0.2971	0.0714	4.15	0.5634	0.0955	5.90	0.6444	0.0918	7.0
		0.3317	0.0784	4.23	0.4611	0.0820	5.62	0.6714	0.0975	6.8
		0.3566	0 0884	4.03	0.4889	0.0980	4.99	0.6778	0.0916	7.4
		0.3030	0 0727	4.16	0.5627	0.1043	5.39	0.6454	0.1207	5.3
	Total.....	1.2884	0.3109		2.0761	0.3798		2.6390	0.3916	
	Mean.....	....	......	4.1			5.4			6.6
Solution 2, high phosphate....	Green weight	2 7163	1.4828	1.83	5.9147	1.5232	3.88	6.3349	1.4203	4.48
		2 8432	1.4268	1.99	5.6276	1.2438	4.52	6.2000	1.1125	5.57
		3.0169	1.5755	1.90	4.4532	1.4182	3.14	7.4203	1.7893	4.14
		2.6996	1.7705	1.50	6.1466	1.4132	4.35	7.5750	1.7514	4.32
	Total.....	11 2760	6.2556		22.1421	5.5984		27.5302	6.0735	
	Mean...	......	.....	1.8			3.9			4.9
	Dry weight	0.2817	0.0920	3.09	0.5610	0.1382	4.06	0.7025	0.1282	5.55
		0.3000	0.0861	3.48	0.5489	0.1215	4.51	0.6587	0.1429	4.60
		0.3220	0.0939	3.42	0.4356	0.1114	3.91	0.7751	0.1574	4.92
		0.2994	0.1054	2.84	0.5776	0.1168	4.94	0.7846	0.1637	4.79
	Total	1.2061	.3274		2.1221	0.4879		2.9409	0.5922	
	Mean.....	....	.. ....	3.2			4.3			4.96

EXPERIMENT II: WHEAT

The duration of this experiment was 29 days. The pH value in case of these culture solutions when prepared was brought in each case to 5.8.

Growth characters.—No apparent differences were noted before or after harvesting in size and color of plants in the cultures;

all showed a healthy green. The high phosphate plants stood more erect and were more rigid, as was noted in case of barley. The results of the three series of this experiment are given in table II, where both dry and green weights show (with one exception) that ratios decrease with the increase of phosphates in the solution. For the 22-day period it will be noticed that there is an increase of 0.2, rather than a decrease, in the ratio for the green weights; but other than this, all remaining figures point in the same direction. The top root ratios based upon dry weights for the three series of this experiment are as follows:

	Solution 1	Solution 2
Series I (15 days)	4 1	3 2
Series II (22 days)	5 4	4 3
Series III (29 days)	6 6	4 96

EXPERIMENT III: COTTON

The solution cultures with cotton were set up in the same way as those of barley and wheat, except that only three plants were set in a quart jar and three jars instead of four were harvested to a series. The duration of this experiment was 48 days. The solutions were changed weekly, and the pH values of the new solutions were found to vary from 5.8 to 6.2; but, as just stated, the two solutions were always brought to the same pH for each renewal.

The line of demarcation between root and stem is not clear in cotton, but by allowing a longer time before beginning to harvest one can determine this with greater accuracy. Two series were conducted with this plant, the first harvested after 40, the second after 48 days. The dry weights only are recorded. The differences in the ratios of tops to roots of plants growing in solution 1 and solution 2 are not striking, but the results are similar to those of experiment I and experiment II, as table III will show.

While the plants growing were healthy looking and green, and apparently the same size, the dry weights showed decidedly greater growth for the low phosphate solutions. There is a possibility that the high phosphate solution used is too near the maximum concentration for the optimum growth for young cotton seedlings. This question is being tested in our laboratory.

EXPERIMENT IV

The three foregoing experiments with barley, wheat, and cotton show unmistakably that the ratio of top to root growth in these plants is affected by the concentration of phosphates in the solution, and that the effect was the opposite of that which has been shown to result from nitrate treatment in increasing quantities. In every case the dry weights showed a decrease in the ratios as phosphates were increased. This was true of green weights also, with a single doubtful exception.

TABLE III

EFFECT OF INCREASING PHOSPHATE CONCENTRATION ON RATIO OF TOP TO ROOT GROWTH IN COTTON SEEDLINGS (DRY WEIGHT IN GRAMS)

		SERIES I (40 DAYS)			SERIES II (48 DAYS)		
		Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Solution 1, low phosphate...	Dry weight	2 2326	0 2164	10 32	2.4462	0.2053	11 92
		1.7854	0 1781	10 03	2.3346	0.2074	11.26
		1 .9440	0 2176	8 93	2.8945	0.2473	11.30
	Total... .	5.9620	0.6121		7.6753	0.6600	
	Mean	......	....	9.76			11.49
Solution 2, high phosphate...	Dry weight	1.5754	0 1639	9 61	1.8073	0.2076	8.71
		1.9432	0 2081	9 34	2.0010	0.2397	8.35
		1.7916	0.2354	7 61	1.9287	0.2308	8.34
	Total..	5.1112	0 6074		5.7370	0.6781	
	Mean	.. ..	.. . ...	8 85			11 13

Since the results found in these experiments indicate greater relative increase of roots, experiment IV was conducted with the aim of determining whether or not this increase is due to the effect of the salt acting directly upon the roots.

ROBBIN'S (3) method of growing root tips in sterile cultures was followed. The seeds (corn only being used) were carefully selected, sterilized in $HgCl_2$ (1–1000) for 45 seconds, washed thoroughly in sterile distilled water, and germinated on sterile agar in 250 cc. wide-mouthed extraction flasks. When the roots of the germinating seeds reached a length of 3 cm. or more they were removed under sterile conditions and transferred to the prepared sterile culture solutions. The pH values of lots of the solutions were tested before and after sterilization and no change was observed. The lengths were carefully measured, after which they were set away in a dark chamber in the greenhouse to grow. Increase in length and number of secondary roots produced were the chief points considered. Other

TABLE IV

EFFECT OF INCREASING PHOSPHATE ON GROWTH OF ROOT TIPS OF CORN UNDER PURE CULTURE CONDITIONS

Solution 1, low phosphate				Solution 2, high phosphate			
Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters	Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters
	Series I (19 days)						
1	3.0	8.5	19 LD*	1	4.0	12.5	25 SD
2	6.0	11.5	106 SD	2	2.0	8.0	18 SD
3	4.5	12.4	45 LD	3	3.0	5.3	5 SD
4	5.0	9.3	68 SD	4	4.0	7.0	42 SD
5	7.0	12.3	100 LD	5	4.5	6.2	14 SD
6	5.5	13.0	71 SD	6	6.0	10.4	16 LD
7	4.0	12.6	75 SD	7	5.0	7.3	51 SD
8	6.0	16.6	134 LD	8	5.0	6.5	12 SD
9	6.0	10.5	76 SD	9	3.5	7.2	18 SD
10	5.0	16.3	85 LD	10	6.0	8.4	65 SD
11	5.5	12.0	73 SD	11	3.0	5.3	7 SD
12	3.5	13.5	60 SD	12	3.0	5.8	9 SD
13	4.5	8.3	17 SD	13	8.0	11.6	56 SD
14	4.5	17.0	54 LD	14	7.0	9.0	62 SD
15	6.5	13.7	124 SD	15	5.5	8.8	60 SD
16	4.0	10.0	51 LD	16	4.5	8.0	27 SD
17	5.0	10.5	55 SD	17	5.5	8.0	63 SD
Total	85.5	208.0	1213		83.5	135.3	550
Average per culture	5.03	12.2	71.35		4.9	7.88	32.3
Percentage increase in length		137				60.8	
	Series II (16 days)						
1	3.5	16.3	45 LD	1	5.0	7.3	34 SD
2	6.3	15.3	71 LD	2	3.5	6.5	33 SD
3	3.3	17.8	37 LC	3	4.5	7.4	14 SD
4	7.0	15.8	97 LD	4	4.0	7.2	19 SD
5	3.6	14.2	96 SD	5	5.3	7.8	45 SD
6	4.7	14.8	82 SD				
7	6.5	14.2	74 SC				
8	3.3	16.8	15 LC				
9	6.5	20.3	89 LD				
Total	44.7	145.5	606		22.3	36.2	165
Average per culture	4.96	16.6	67.3		4.46	7.24	29
Percentage increase in length		234.6				62.3	

TABLE IV—*Continued*

SOLUTION 1, LOW PHOSPHATE				SOLUTION 2, HIGH PHOSPHATE			
Culture no	Initial length in cm.	Final length in cm.	No of secondary roots and growth characters	Culture no	Initial length in cm.	Final length in cm	No. of secondary roots and growth characters
	Series III (16 days)						
1	5 2	11 5	58 SD	1	3 5	5 4	20 SD
2	3 8	7 0	21 SD	2	6 0	7 2	67 SD
3	5 2	13 1	69 SD	3	7 8	9 6	49 SD
4	6 0	15 8	83 LD	4	3 5	5 0	25 SD
5	9 2	16 1	115 SD	5	3 3	5 1	20 SD
6	4 5	12 4	99 SD	6	6 8	9 0	63 SD
7	5 2	14 7	58 LD	7	6 2	7 3	51 SD
8	7 6	16 3	48 LD	8	7 6	11 0	91 SD
9	3 8	15 0	61 SD	9	4 6	7 3	21 SD
10	6 0	13 4	61 SD	10	8 3	14 0	101 SD
11	6 6	11 0	65 SD	11	4 8	6 5	23 SD
12	3 4	13 4	44 LD	12	4 0	5 2	22 SD
Total	66 5	159 7	782		66 4	92 6	553
Average per culture	5 5	13 3	65 1		5 5	7 7	46 0
Percentage increase in length	..	141 8				40	

* L (long) denotes secondary roots greater than 0 75 cm., D denotes uniform distribution of secondary roots along growing tip; S (short) denotes secondary roots, equal to or less than 0 75 cm , C (crowded) denotes tufting of secondary roots around cut end

growth characters were noted, including the manner of growth of the secondary roots, whether long or short, and crowded toward the cut ends or distributed throughout the length of the root.

Three series of these pure culture experiments were conducted, running for 19, 16, and 16 days respectively, as shown in tables IV. It will be seen that the number of cultures which figure in the results is not the same for the different series. In series II the number is smaller, as several cultures had to be discarded because of contamination. In series I (17 cultures) the average length of root tips in the low phosphatic solution at the beginning was 5.03 cm.; at end of 19 days it was 12.2 cm. The increase in length during this period was 133 per cent. The average number of secondary roots was 71.35.

For the seventeen cultures in the high phosphate solution the average initial length was 4.9 cm., which increased to 7.88 cm. in 19 days, or 57.9 per cent. Also, for this first series the average number of secondary roots, which was found to be 71.35 per culture for the low phosphate solution, was only 32.3 for the high. In series II we have not the same number of cultures in the solutions for comparison (nine cultures in solution 1 and five in solution 2), but, with this reservation, we find the average of results similar to those for series I. In series III we have a still closer similarity of results to those of series I. As one studies the three series in table IV, it becomes clear that under the conditions of this experiment, increasing the concentration of phosphates in the nutrient solution neither brings decreased growth in length nor increase in the number of secondary roots. The growth features of the sterile cultures showed no marked differences. Strikingly long secondary roots occurred seldom in high phosphate cultures, but were of frequent occurrence in the low phosphate.

Discussion

It is well known that phosphates play varying rôles in the living plant. Their beneficial effects upon underground portions of certain plants growing under field conditions have been observed for nearly a century, but there is little to be found in the literature to indicate that these field results have been followed up by laboratory experiments with sufficient definiteness to determine the exact way in which phosphates function in the plant. The water culture experiments reported here for barley, wheat, and cotton (tables I–III) show without question that the ratios of top to root growth decrease in each case as the phosphate concentration of the solution increases. One may interpret such a result to mean either that the salt has a retarding effect upon the tops or a stimulating effect upon the roots. LAWES and GILBERT stated many years ago: "It is certainly true that it (super-phosphate of lime) causes a much inhanced development of the underground collective apparatus of the plant especially of lateral and fibrous roots." Sir JOHN RUSSEL (4) in 1921 wrote: "In the absence of phosphorus, swedes and turnip roots will not swell, but will remain permanently dwarfed." These quotations, the old and the new, point to some direct effect of the salt upon the underground parts as the explanation.

The pure culture experiments reported in the three series of table IV are strikingly constant in showing that increasing the phosphate concentration not only does not have the effect of stimulating directly growth in length or multiplication of lateral roots, but that both of these are retarded under such conditions. Corn root tips only have been dealt with so far in these experiments. A summary of table IV shows the results more clearly.

The experiment shows for example that cellular activity which should manifest itself in increased growth in length or multiplication of secondary roots is not increased by direct application of phosphates, as is implied in the usual statements. The actual fact noted, therefore, that there is a decreasing ratio of tops to roots as the phosphate concentration is increased, must find explanation in the formation of compounds or simple substances in connection with photosynthetic activity in the tops, which are translocated to the roots and manifest themselves there by their growth stimulating or storage effects.

TABLE IV—SUMMARIZED

Solution 1			Solution 2	
	Percentage increase in length	Average no. secondary roots per culture	Percentage increase in length	Average no. secondary roots per culture
Series I, 19 days.	133	71.35	57 9	32.3
Series II, 16 days.	234	67.3	62.3	29
Series III, 16 days.	141.9	65.1	40	46

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EFFECT OF NITRATE SALTS UPON GROWTH AND COMPOSITION OF TOBACCO LEAVES[1]

A. R. C. HAAS

(WITH ONE FIGURE)

Nitrate fertilization of tobacco has been shown by VALLEAU and JOHNSON (10) to be efficacious in bringing about the recovery of tobacco leaves from frenching. The writer has been able to produce chlorotic-appearing tobacco leaves in experiments by using 10-gallon containers filled with Sierra loam which was known to contain a very small concentration of nitrate. VALLEAU and JOHNSON were able to bring frenched plants back to normal growth in soil to which was added a complete nutrient or nitrogen as sodium, potassium, calcium nitrate, or ammonium sulphate, etc.

It seemed of interest to consider solely the effect of the cation added to the soil when the nitrate salt was used in each case. Ammonium, sodium, potassium, calcium, and magnesium nitrate were used respectively for the various soil cultures. Each day the soils were given 3 liters of their respective nitrate solutions, made up with distilled water, the nitrate concentration in each case being about 2000 p.p.m. After several weeks of such applications, during which time and subsequent thereto the replaced bases were free to escape in the drainage water, the concentrations of the solutions applied were reduced gradually. At the time (July 8) of transplanting the young Kentucky broad-leaf tobacco plants from the seed bed to the experimental containers, the solutions applied contained about 500 p.p.m. of nitrate. In this manner the soil was changed considerably in its base exchange complexes, and yet with but one exception moderate growth was still permitted. In the sodium nitrate-treated soil the plants were able to maintain slight growth if they were raised on little mounds of soil at the time of being transplanted. By doing this none of the black surface solution, which drained away slowly, came into direct contact with the tender portions of the plant. Notwithstanding these precautions the plants in the sodium nitrate-treated soil grew so slowly that they were excluded from the experiment. Tobacco plants were transplanted into some untreated Sierra loam soil as a control and received only distilled water. One such control was given the sodium nitrate treatment in order to give the plants opportunity to become well established prior to the occurrence of severe alterations in the nature of the base exchange complex. In spite of the large and repeated applications of solution, the growth was excellent. There was also a moderate growth in the other treated soils.

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[1] Paper no. 195, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The most important effect observed was in the leaves of the plants in the ammonium nitrate-treated soil. Yellowish areas occurred between the veins, as shown in fig. 1. This was first noticeable in the lowest leaves, and progressively made its appearance in leaves higher up as they became mature. The yellow areas were generally distributed throughout the entire length of the blade, and gave the leaves a mottled appearance. This differs from the chlorosis that GARNER, MCMURTREY, and MOSS (2) have shown to result from a magnesium deficiency in which the chlorosis begins at the tip and along the outer margins of the oldest tobacco leaves and advances toward the leaf base (cf. JOHNSON 4). The effect produced upon the leaves by the ammonium nitrate treatment of the soil also differs from that produced by a deficiency of potassium, as described by MOSS, MCMURTREY, LUNN, and CARR (6), in which the leaves are puckered and rough, with mottling beginning at the tips, often followed by the appearance of small centers of dead tissue.

Further differences between the effects of ammonium and that of a deficiency of magnesium or potassium are obtained by analyzing the leaves of the tobacco plants taken from the variously treated soils. The most mature leaves of the plants in the various cultures were collected on November 23, dried, and ashed. Table I shows the analyses of the ash of the various leaf samples.

It will be seen that for the plants grown in the soil treated before the planting was made, the ash, as a percentage of dry matter, is greatest in the control plants. The sodium nitrate treatment of the soil was not begun until after the planting was made, and the percentage of ash in this case was slightly greater than that of the control plants. After the base exchange complex of the soil has been

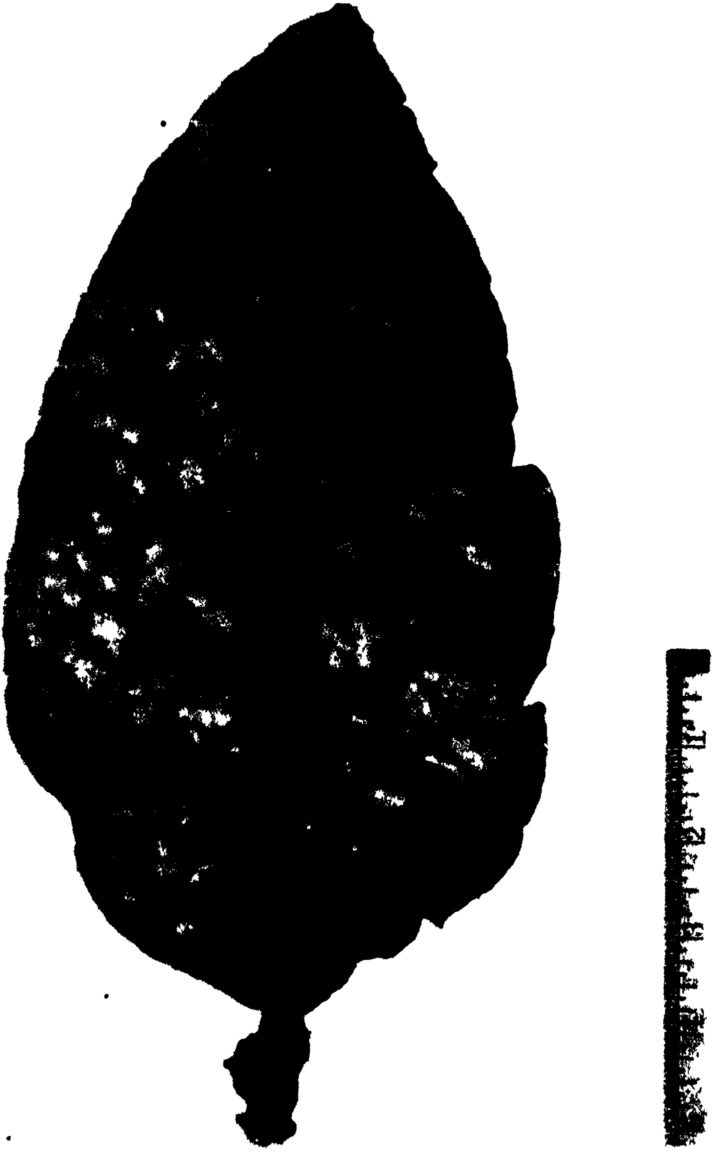

FIG. 1.—Tobacco leaf from plant grown in ammonium nitrate-leached soil

acted upon by a single salt solution until replacement and leaching away of the replaced bases has progressed to a considerable degree, the ash of the leaves, as a percentage of the dry matter, becomes lower than that of the control. When saturation of the base exchange complex of the soil with a single base is approached, deficiencies of various bases no doubt may enter as factors in the growth of the plants.

The calcium, as a percentage of the dry matter, was greatest in the leaves of the plants from the sodium nitrate-treated soil, followed in order by the calcium nitrate-treated, and the control; whereas the ammonium, potassium, and magnesium-treated showed a marked reduction below that of the control. The low value in the one case of calcium nitrate is due to the yellowing and drying up of the mature lowest leaves, and hence is not strictly comparable with the other cultures in this regard. The marked reduction in the percentage of calcium in the dry matter of the leaves of the plants from the ammonium, potassium, and magnesium-treated soil as compared with the control is very striking. This is no doubt a result of the replacement of the calcium from the base exchange complex of the soil by the base of the added salt solution, and the subsequent leaching out of the replaced calcium and other bases in the drainage water.

TABLE I

ASH COMPOSITION OF TOBACCO LEAVES IN RELATION TO SOIL TREATMENT

SOIL TREATMENT	ASH AS A PERCENTAGE OF DRY MATTER	ASH CONSTITUENTS							
		Percentage of dry matter (calculated from ash analyses)				Percentage of ash			
		Ca	Mg	Na	K	Ca	Mg	Na	K
Sodium nitrate	18.85	4.45	1.50	1.41	1.59	23.59	7.98	7.47	8.42
Calcium nitrate	11.02	2.87	0.29	0.34	1.58	26.07	2.65	3.07	14.34
Magnesium nitrate	7.99	0.78	1.93	0.47	1.01	9.79	24.10	5.85	12.64
Ammonium nitrate	5.55	0.78	0.30	0.19	1.43	14.11	5.46	3.47	25.78
Ammonium nitrate	7.85	0.99	0.32	0.65	1.83	12.60	4.05	8.27	23.32
Control (distilled water)	18.39	3.34	0.55	0.59	3.62	18.16	3.00	3.20	19.68
Calcium nitrate	15.46	3.50	0.32	0.67	2.87	22.66	2.07	4.34	18.55
Potassium nitrate	16.68	0.86	0.28	1.63	7.58	5.16	1.69	9.79	45.47

The values of the magnesium, as a percentage of the dry matter, are not very great even for the plants in the magnesium-treated soil. There was a reduction below that of the control in the plants in the calcium, ammonium, and potassium-treated soil, but an increase over that of the control in the plants in the sodium nitrate-treated soil. Notwithstanding the large additions of sodium nitrate to the soil, the leaves contained relatively little sodium, since as large a percentage of sodium was found in the dry matter of the leaves of the plants in the potassium-treated as in the sodium-treated soil. All treatments decreased the percentage of potassium in the dry matter as compared with the control, with the exception of the plants in the potassium-treated soil where a large increase was noted

The calcium, as a percentage of the ash of the leaves, is reduced by the potassium, magnesium, or ammonium treatment. The dry matter of the leaves of the plants in the magnesium-treated soil contained a relatively low percentage of magnesium, but because of the small percentage of ash in the dry matter, the percentage of magnesium constitutes about one-fourth of the ash. The ash of the plants in the potassium-treated soil contained over 45 per cent of potassium. Of the cultures receiving no potassium, the ash of the leaves from the sodium nitrate-treated soil contained the least, and those from the ammonium-treated the largest percentage of potassium.

Having considered the effects that the added bases in the nitrate solution had upon the composition of the leaves, the effects observed in the leaves of the plants in the ammonium nitrate-treated soil can hardly be due either to a deficiency of magnesium or potassium, but appear to be an effect of the ammonium base of the added solution. For if we consider the magnesium as a percentage of dry matter, we find that the results for the leaves of the plants in the calcium- and in the potassium-treated soil are as low as those of the ammonium-treated, and yet have none of the leaf symptoms as found in the ammonium-treated. The magnesium as a percentage of ash is higher in the leaves of the plants from the ammonium-treated soil than in all but the sodium and magnesium-treated. Consequently, the effect produced on the plants in the ammonium-treated soil cannot be attributed to magnesium deficiency or "sand drown" (1).

If we consider the percentage of potassium in the dry matter, the values found for the leaves of the plants in the ammonium-treated soil are not unlike those found for the leaves of the plants in the sodium, calcium, or magnesium-treated soil where no effects were observed. The potassium, as a percentage of ash, is greater in the leaves of the plants from the ammonium-treated soil than in the leaves of the plants on any other treated soil except the potassium-treated, and, therefore, a deficiency of potassium cannot be regarded as the cause of the symptoms observed in the leaves of the plants in the ammonium-treated soil (6). It is concluded, therefore, that when the base exchange complex of the soil is largely saturated with the ammonium base, the soil solution in equilibrium with the nearly saturated ammonium soil complex becomes toxic to the tobacco leaves and produces the effects observed. The toxic effect of ammonium ions for certain plants has been shown by SÖDERBAUM (7, 8, 9).

It is of interest to note that none of the leaves of the plants in the nitrate-treated soil showed frenching. This known relationship, and the observations of MORRIS (5) that legumes when plowed under have brought about the recovery of rosetted pecan trees, have led VALLEAU and JOHNSON (10) to suggest that the frenching of tobacco leaves and the rosette of pecan trees have a common cause, namely, a deficiency of available nitrogen. There is, however, no experimental evidence to show that the plowed-under legumes brought about the recovery as a result of the nitrogen in the legumes. MORRIS in fact showed that applications of sodium nitrate, dried blood, or manure gave no improvement. HAAS, BATCHELOR, and THOMAS (3) have reported severe cases of rosette of pecan trees in experimental plots at the Rubidoux tract of the Citrus Experiment Station, where the sources of nitrogen were sodium nitrate, dried blood, and steamed bone meal.

When previously untreated Sierra loam soil was placed in two wide sewer pipes placed one above the other, and planted to pecan trees and leached every day with potassium and magnesium nitrate solution respectively, severe cases of pecan rosette developed. In these unpublished results on pecan trees and on the mottling of citrus, the writer finds these physiological diseases to be independent of the nitrogen portion of the added salt solution.

Summary

1. Frenching of tobacco leaves occurs when nitrate is deficient but disappears upon the application of sufficient nitrogen to the soil.

2. The continual application of nitrate solutions to soil prior to and during the growth of tobacco plants affects the base exchange complex in the soil, and consequently the bases absorbed by the leaves. Such continual applications of ammonium nitrate bring about toxic conditions in the tobacco leaves.

3. The recovery of tobacco from frenching by the application of nitrate does not appear to bear any direct relationship to the recovery of pecan trees from rosette following the use of legumes.

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STUDIES IN CALIFORNIAN HEPATICAE

II. FOSSOMBRONIA LONGISETA

ARTHUR W. HAUPT

(WITH PLATE VII)

In a former investigation of *Fossombronia cristula*, a species ranging from New England to Indiana, the writer (3) found several features in disagreement with HUMPHREY'S (4)[1] earlier study of *F. longiseta*, a form widely distributed in California. The chief point of variance in the two papers concerns the antheridium, HUMPHREY describing a unique development allying *Fossombronia* with *Sphaerocarpus* and *Geothallus* rather than with the other Jungermanniales. The writer, on the other hand, found the development of the antheridium in no way peculiar, but like that characteristic of other members of the order, as LEITGEB (5) had previously reported for *Fossombronia pusilla*.

With respect to the development of the antheridium, the Marchantiales and Jungermanniales stand in striking contrast to each other, and yet within each order this development is remarkably uniform, as is well known. Consequently to find within a single genus a wide variation in regard to this fundamental phase of the life history would indeed be an extraordinary circumstance. The present study of *Fossombronia longiseta* was undertaken particularly to attempt to determine whether the development of the antheridium in this species is really unique, as reported by CAMPBELL and by HUMPHREY, or whether it is like that of *Fossombronia cristula* previously described by the writer. Incidentally certain noteworthy details concerned with the development of the archegonium and of the embryo were discovered, and these are included in the present paper. No attention is given to such other phases of structure and development as are adequately described in the literature cited.

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[1] In the 1905 edition of CAMPBELL'S *Mosses and ferns* is found a brief statement regarding the development of the antheridium of *Fossombronia longiseta*, a short description of the early stages in embryogeny, and three sets of figures drawn for the book by HUMPHREY showing the development of the antheridium, archegonium, and embryo. HUMPHREY'S own paper (4) did not appear until the following year, and so to CAMPBELL should belong credit for the first published account of the development of this species.

Material

Most of the material used in this investigation was obtained during 1925–1928 from several different localities in the San Gabriel Mountains of Los Angeles County, chiefly from Santa Anita Canyon. One collection was also made in 1928 at the foot of Bridal Veil Falls in Yosemite Valley. So far as the features dealt with in this study are concerned, no differences could be observed between the plants collected in Yosemite Valley and those from southern California. The only figure drawn from the Yosemite material is fig. 2.

The most favorable material to illustrate the development of the sex organs was collected in November and December. Like other liverworts, the plants dry up during the long rainless summer. Collections were also made throughout the winter and early spring, but young stages in the development of the sex organs were more difficult to find at this time than in the late autumn.

Sex organs

Fossombronia longiseta is monoecious, the antheridia and archegonia being more or less separately grouped. Frequently they are somewhat intermixed, however, a feature which makes it very difficult to distinguish between the two kinds of sex organs when young. In general there are relatively more antheridia produced when the plants are young and relatively more archegonia later. The sex organs develop acropetally from the dorsal segments of the dolabrate apical cell, but occasionally they may arise farther back among the older ones.

ANTHERIDIUM

The antheridium develops from a papillate initial which undergoes a transverse division into a large basal cell and a smaller outer cell. As in *Fossombronia cristula*, another transverse wall appears, probably in the outer cell, in agreement with other members of the order. In *Pallavicinia* the writer (2) has demonstrated this exact sequence of wall formation in the young antheridium. Of the three cells present, the upper one is the primary antheridial cell, the middle one forms the stalk, and the lower one remains imbedded in the thallus (fig. 1).

The primary antheridial cell soon undergoes a median vertical division, and the primary stalk cell usually divides at the same time, either vertically (figs. 1, 2) or transversely (fig. 4). The first vertical division of the primary antheridial cell is not followed by another vertical one at right angles to the first, as described by HUMPHREY (4); but four periclinal walls are formed in the primary antheridial cell, arranged as shown in fig. 3. The young antheridium now consists of an imbedded portion, a stalk, and two spermatogenous cells surrounded by four primary wall cells, exactly as in *Fossombronia cristula* and other anacrogynous Jungermanniales. In no case were periclinal walls seen in the second tier of cells, another feature described by HUMPHREY. The two primary spermatogenous cells stain more deeply than the cells of the wall and stalk, and thus are easily recognized. Further growth of the antheridium is typical in every way, and need not be described.

HUMPHREY'S account of the development of the antheridium of *Fossombronia longiseta* differs from that described here in two main respects: (1) The primary antheridial cell and the cell just below it divide by vertical walls arranged at right angles to each other, resulting in the formation of an octant. (2) Periclinal wall formation, differentiating the primary spermatogenous cells from the sterile jacket, involves the entire superficial portion of the young antheridium, that is, two tiers of cells. A complete discussion of HUMPHREY'S account is given in the writer's paper on *Fossombronia cristula* (3), and nothing more need be said here than that the present study has yielded no evidence whatsoever in support of his interpretation of the development of the antheridium.

ARCHEGONIUM

Like the antheridium, the archegonium arises from a papillate initial usually formed very close to the apical cell. A transverse division results in the formation of a large basal cell and a smaller outer cell (fig. 5), although HUMPHREY finds that the outer cell is considerably the larger. HUMPHREY also states that the archegonium initial does not extend so far above the surrounding cells as does the antheridium initial, but no evidence of this was seen by the writer. The basal cell may divide again transversely, or it may remain undivided until three vertical walls have appeared in the outer cell in the way characteristic of all Hepaticae (figs. 5, 6). HUMPHREY reports that the basal cell does not undergo another division until after the vertical walls have appeared in the outer cell. This is certainly not invariably the case. The behavior of the basal cell, as here reported, corresponds exactly with that of *Fossombronia cristula* previously described by the writer (3).

The division of the primary axial cell to form a cover cell and a central cell (fig. 7), and the formation by the latter of a primary neck canal cell and a ventral cell (fig. 8) are in every way typical. The primary neck canal cell soon gives rise to an upper and a lower cell (fig. 8). Both of these may divide again (fig. 11), the upper one dividing first (figs. 9, 10). More frequently, however, the lower neck canal cell may fail to divide. The cover cell divides by a vertical wall most commonly soon after the division of the primary neck canal cell has taken place. The ventral cell now forms the ventral canal cell and the egg (fig. 12), while the two upper neck canal cells may give rise to four (fig. 13).

Of the two cells derived from the primary neck canal cell, the lower one may remain undivided while the upper one divides once before the division of the ventral cell. This results in an archegonium with three neck canal cells (fig. 12). In other cases the lower cell resulting from the division of the primary neck canal cell may remain undivided while the upper one divides twice, thus resulting in an archegonium with five neck canal cells (fig. 13). Sometimes the lower cell divides once and the upper one twice, thus producing six neck canal cells (fig. 15). Various intermediate conditions may occur (fig. 14).

In *Fossombronia cristula* the writer found that 6–8 neck canal cells are formed. In *F. longiseta*, HUMPHREY states that "the number of neck canal cells is, so far as observed, invariably six." Since his fig. 44 represents an archegonium with an egg, ventral canal cell, and two neck canal cells, and his fig. 45 shows one with six neck canal cells, it is evident that the number may be increased after the ventral canal cell and egg are differentiated. HUMPHREY does not, however, refer to this fact.

Embryo

While no attempt was made to work out a complete series of stages in the development of the embryo, enough stages were seen to indicate that the embryogeny of *Fossombronia longiseta* is essentially the same as described by CAMPBELL (1) and HUMPHREY (4). Most of the embryos observed consisted of four tiers of cells, each tier being composed of two or four cells. In *F. cristula* the writer (3) found 6–8 superimposed cells before the appearance of the vertical walls. Thus in *F. longiseta* the vertical divisions occur much earlier.

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EXPLANATION OF PLATE VII

The magnification in each case is 500 diameters

FIG. 1.—Two young antheridia, the one to left consisting of basal cell, stalk cell, and primary antheridial cell; the one to right shows periclinal wall formation in antheridial cell and division of basal and of stalk cells by vertical walls.

FIG. 2.—Slightly older antheridia; in one to right the primary wall cells have divided, in other one the primary spermatogenous cells have increased to four; in both cases the basal cell has remained undivided.

FIG. 3.—Cross-section of young antheridium showing two primary spermatogenous cells surrounded by four primary wall cells.

FIG. 4.—Young antheridium showing formation of stalk.

FIG. 5.—Two young archegonia; the one to left shows division of initial to form large basal cell and smaller outer cell; in one to right a horizontal wall has appeared in basal cell and vertical wall in outer cell.

FIG. 6.—Formation of vertical wall in stalk and of second vertical wall in outer cell.

FIG. 7.—Formation of cover cell and central cell from primary axial cell, central cell about to divide.

FIG. 8.—Formation of primary neck canal cell and ventral cell from central cell, primary neck canal cell undergoing first division.

FIG. 9.—Division of cover cell by vertical wall and formation of three neck canal cells, lower one not yet divided.

Fig. 10.—Later stage showing division of lower neck canal cell.

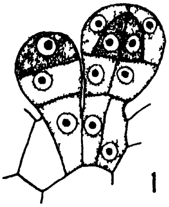
FIG. 11.—Young archegonium with axial row consisting of ventral cell and four neck canal cells.

FIG. 12.—Division of ventral cell to form ventral canal cell and egg, lower neck canal cell undivided.

FIG. 13.—Archegonium with lower neck canal cell still undivided, upper one having divided twice.

FIG. 14.—Archegonium with lower neck canal cell undivided, upper one having formed three.

FIG. 15.—Archegonium in which the lower neck canal cell has divided once, upper one twice.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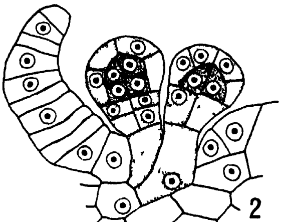
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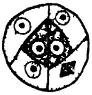
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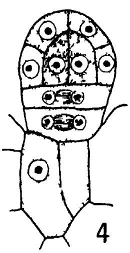
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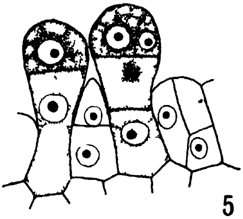
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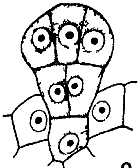
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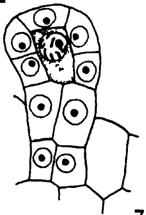
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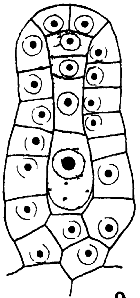
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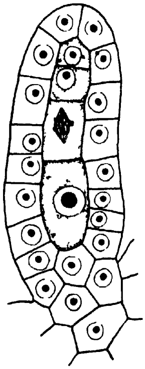
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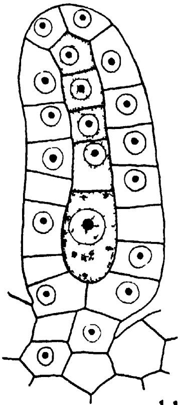
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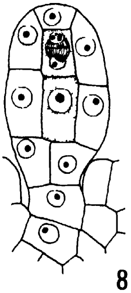
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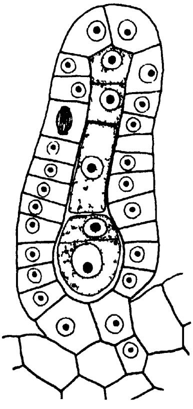
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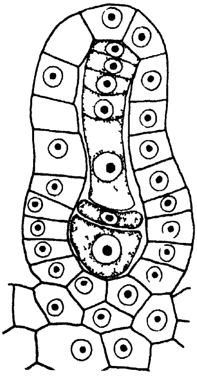
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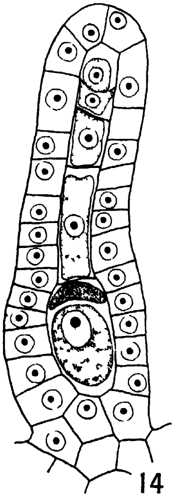
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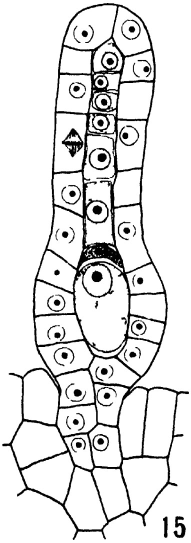
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HAUPT on HEPATICAE

BRIEFER ARTICLES

A CASE OF PHYLLODY IN YUCCA ELATA

(WITH TWO FIGURES)

Yucca elata Engelm., an evergreen of the family Liliaceae, occurs abundantly over the grasslands of southern New Mexico. In the late spring, after favorable precipitation, the plant produces a long flower stalk, surmounted by a great cluster of white or cream colored flowers, as shown in fig. 1. In observations made on the Jornada Range Reserve, a grazing experiment station in southern New Mexico conducted by the United States Forest Service, practically every plant of *Yucca elata* flowered during May or June of 1926. Later in that summer, several flower stalks were found with leaf clusters instead of flowers. In the summer of 1928 the excellent specimen of phyllody shown in fig. 2 was found on the Reserve. All the cases of phyllody have been found from one to two months after the regular flowering season for this plant. Observations to date have shown that such unusual flower stalks, as well as the ordinary ones, do not persist in a green condition longer than a year after production.—R. S. CAMPBELL, *Jornada Range Reserve, U.S. Forest Service, Las Cruces, New Mexico.*

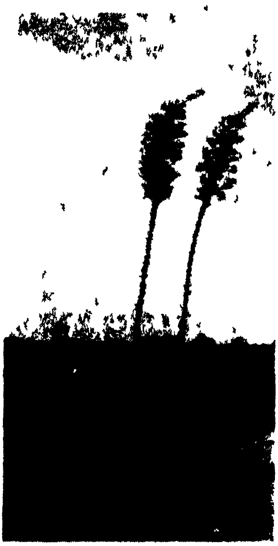

FIG. 1.—*Yucca elata* in full bloom

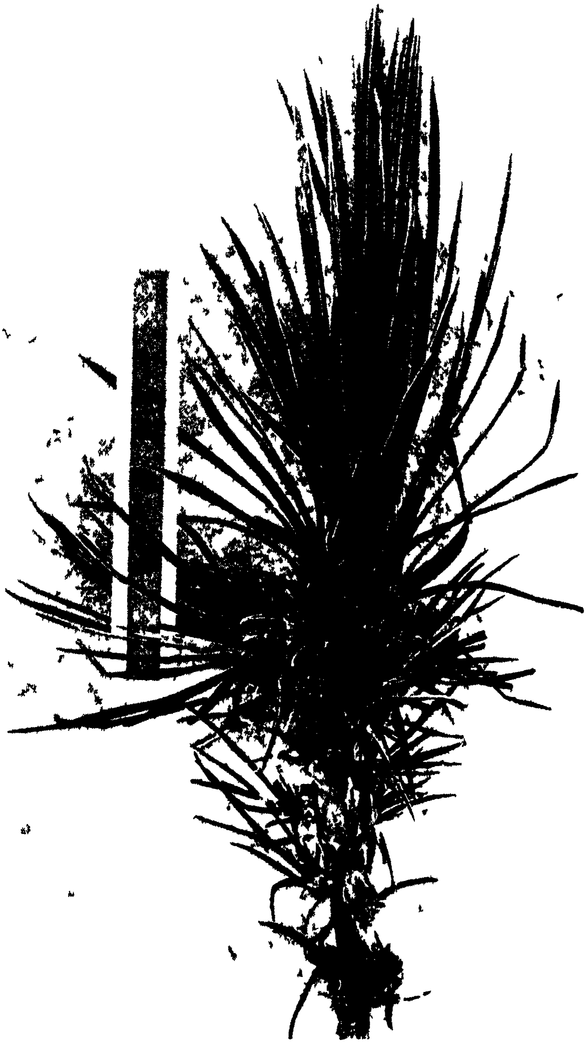

FIG 2 —Phyllody in *Yucca elata*

CURRENT LITERATURE

BOOK REVIEWS

A moss flora of North America

American moss students have been at a considerable disadvantage in recent years because of the lack of an up-to-date general manual. LESQUEREUX and JAMES's *Manual of thc mosses of North America* (1884), supplemented by the BARNES and HEALD *Analytical keys to the genera and species of North American mosses* (1896), has long been out of date and difficult to obtain. Of the *North American flora,* only two parts have appeared dealing with mosses (1913), covering only nine families. This last work has no illustrations, is brief and rather technical, and because of its wide range (including all North America and the West Indies with many tropical mosses) the keys are larger and rather more difficult to use.

To supply the need for a general and not too difficult manual of all the mosses of the United States and Canada, GROUT, author of *Mosses with a hand-lens* and *Mosses with hand-lens and microscope,* has begun the publication of a moss flora of North America, north of Mexico. Part I of Volume 3 has appeared[1] (September 1928), including the Climaceae, Porotrichaceae, Isotheciaceae, and the Brachythecieae under the Hypnaceae. The families and genera are fully described, and there are keys to the genera and to the species. For each species there is given the more important synonymy, a full but not too briefly technical description, type locality, habitat, geographical range, citations to illustrations and to exsiccati, and usually critical and comparative notes. Varieties are described and line-drawn plates are included of all the mosses that have not been already illustrated in the author's *Mosses with hand-lens and microscope,* with which the moss flora agrees in size of page. The taxonomic sequence followed is that which GROUT has outlined in The Bryologist (31:56–61. 1928), and which differs considerably from that of the second edition of *Die natürlichen Pflanzenfamilien* (Band 10, Musci, 1. Hälfte, 1924, and Band II, 2. Hälfte, 1925).

This is an excellently and carefully prepared manual, the value of which is considerably enhanced by the good illustrations, and by the very interesting and helpful comparative and critical notes which accompany most of the specific descriptions. The reviewer ventures the opinion that the sequence used by GROUT will find favor with many American bryologists. Great service will be done American bryology by the publication of this manual, and it is to be hoped that it may be rapidly completed.—O. E. JENNINGS.

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[1] GROUT, A. J., Moss flora of North America, north of Mexico. Vol. III, Pt. I. pp. 1–62. *pls. 1–14.* Published by the author, 1 Vine Street, New Brighton, Staten Island, New York City. 1928.

Manual of histochemistry

As a method of attacking the problems of chemical constitution of cells and tissues, histochemistry or microchemistry is recognized as an indispensable aid. It is also used in detecting the presence of soluble constituents in drug materials, and is rapidly becoming useful in the detection of the origin, transformation, and distribution of certain substances in the organism throughout life. Physiologists and pharmacologists need this discipline in much of their research.

To meet the need of a laboratory manual in this field, KLEIN[2] has prepared a little book which will be quite useful to the practicing histochemist. It does not in any sense take the place of such research literature as TUNMANN'S *Pflanzenmikrochemie*, or MOLISCH'S *Mikrochemie der Pflanze*, but it offers to students an introduction to the methods and purposes of microchemistry, with exercises in training for such work. Such a manual has long been needed.

The introductory chapter deals with the significance of histochemistry, the problems, the limits of its service, localization, the materials, reagents, instruments and utensils needed, hints on various types of reactions, histochemical and special methods. The second section gives tests for the inorganic substances, nine cations, and as many anions. Part III deals with the determination of organic substances, aliphatic, cyclic, nitrogenous, glücosidic, pigments, enzymes, membrane substances, and special inclusions of the cell. The final division, only seven pages, considers animal histochemistry. The directions are clear, and suggestions of appropriate plants for study are numerous. The student is aided by 64 text figures, mainly of crystals and other visible features of the histochemical reactions. It is recommended to students and instructors of students in microchemistry as an inexpensive laboratory guide in this important field of plant physiology.—C. A. SHULL.

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[2] KLEIN, G , Praktikum der Histochemie 8vo pp v+71. Berlin: J. Springer, 1929

VOLUME LXXXVIII NUMBER 2

THE

BOTANICAL GAZETTE

October 1929

TOXIC EFFECT OF BORON ON FRUIT TREES[1]

A R C HAAS

(WITH THIRTEEN FIGURES)

It is now well established that small concentrations of boron are indispensable to the growth of certain, if not all plants, and that toxicity results when these small concentrations are slightly increased. Several investigators have recently reviewed the literature bearing on the relation of boron to the growth of plants, and therefore it will be unnecessary in this paper to enter into a discussion of the literature. KELLEY and BROWN (4) have shown that boron occurs in certain soils and irrigation waters of southern California in amounts sufficient to cause severe injury to citrus and walnut trees. The writer has made a study of the toxic effects of boron under controlled conditions, and has produced symptoms in the leaves of certain fruit trees similar to those found in certain orchards of California.

It is of interest at the outset to determine the lowest concentration of boron that will cause injury to citrus seedlings. When grown in the same culture jars, lemon seedlings were found to be more sensitive than orange seedlings to the effects of boron. Unless otherwise stated, boric acid was the source of boron used in all experiments. At a concentration of 1 p.p.m. of boron there was slight injury to all of the lemon leaves and the new leaves became yellow, while the orange leaves were unaffected. At a concentration of 2 p.p.m. all of the lemon leaves showed injury but again the orange leaves showed no effects. Injury first became noticeable on orange leaves when the nutrient solution contained 3 p.p.m. of boron, and then only after the seedlings had been grown in the solution for at least 2 months with frequent renewal of the culture solution.

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[1] Paper no. 201, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California

After 2 months' growth in Hoagland's solution to which boron was added, only 2 out of 25 sweet-orange seedlings showed injury at a concentration of 3 p.p.m. of boron, while with 6 p.p.m. every plant showed injury.

Sweet-orange and lemon seedlings were grown in the same culture jars. Hoagland's nutrient solution was used, to which 10 to 40 p.p.m. of boron was added in the form of manganese borate (MnB_4O_7). In every case injury was produced. Although manganese borate is usually considered to be insoluble, its low solubility is sufficient to produce injury in citrus trees. BRENCHLEY and WARINGTON (1) found that the addition of the so-called insoluble borates to culture solutions sufficed to supply boron in quantities adequate for growth.

The writer has grown lemon and Valencia orange trees budded on sour-orange stocks in dust and rain-protected sand cultures without the addition of boron, and after 3 years the leaves when analyzed by Mr. S. M. BROWN, Assistant Chemist in the Department of Agricultural Chemistry of this Station, showed about 50 p.p.m. of boron in the dry matter. KELLEY and BROWN (4) have shown this concentration to be typical of normal trees when grown in California. As the trees were planted bare-rooted, using only the larger roots and a portion of the trunk, it must be assumed that either sufficient boron gained entrance to the sand in the form of dust in spite of the protection, or that a small amount of comparatively insoluble borate occurred in the original sand and that this maintained a supply of boron adequate for the growth of leaves. A more successful method of reducing the content of boron in the trees may possibly be found by the use of water cultures extending over a period of years. Studies are now under way regarding methods of growing citrus for long periods in water culture, and these may afford suggestions in this direction.

A Eureka lemon and a Valencia orange tree, each budded on sour-orange roots, were grown for 3 years in tanks of sand 4 feet in diameter and 5 feet deep, during which time they were supplied with Hoagland's culture solution. Each tree was irrigated with 432 liters of culture solution containing 25 p.p.m. of boron, and this was followed by Hoagland's solution without boron. The lemon tree was the first to show injury. The leaves burned along the margins and soon abscissed. The new leaves that developed later were very large, thin, and pale yellowish green along the outer portion, but a darker green near the midrib. They were also badly crinkled (fig. 1) and abscissed without becoming mature. The Valencia orange leaves were at first dark green, but, as a consequence of the boron treatment, they became somewhat mottled between the veins near the tip. The yellow areas enlarged, until the leaves finally became almost entirely chlorotic (fig. 2). These leaves fell prematurely. The succeeding leaves were thin in appearance and of a pale yellowish green color, but showed no tendency to crinkle, as did the lemon leaves. In this connection the results of WARINGTON (6) are of interest, in that she found boron injury to be accompanied by chlorosis.

FIG. 1.—Right, normal lemon shoot bearing healthy leaves; left, shoot from lemon tree in sand culture that received boron, bearing burned mature leaves and pale yellowish green and crinkled young leaves.

The preceding results were confirmed by experiments with 2-year old, budded lemon trees grown in sand cultures in containers 20 inches in diameter and 26 inches deep. Hoagland's solution, to which 25 p.p.m. of boron was added, was used. The leaves became somewhat mottled and burned, and the new leaves were thin and crinkled, as in fig. 1.

When such cultures were washed free from the excessive boron before the injury became too pronounced and were then given unmodified Hoagland's solution, the new cycles of growth were badly crinkled, but the later cycles were normal in appearance. These results, as well as those obtained in soil cultures, indicate that, unless the trees are badly injured by boron and have

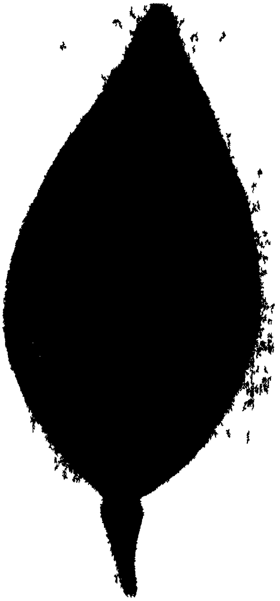

Fig 2

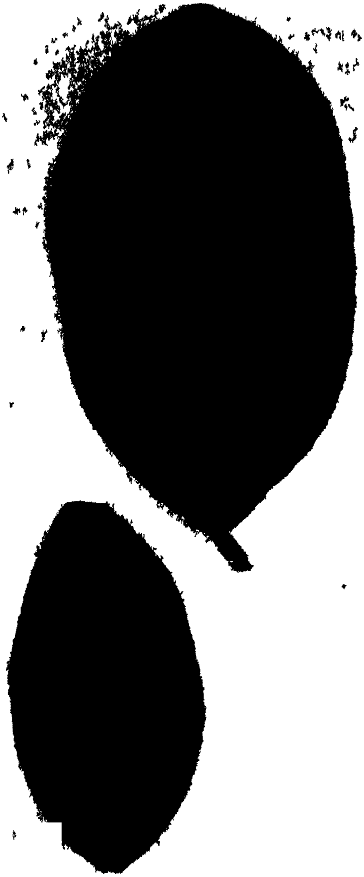

Fig 3

Fig 2 —Toxic effect of boron on leaves of Valencia orange tree grown in sand culture advanced case of chlorosis with slight bronzed coloration of yellow areas

Fig 3 —Toxic effects of boron upon leaves of lemon trees in sand culture: upper, dorsal surface of leaf showing destruction of chlorophyll; lower, ventral surface of leaf showing dark, slightly elevated, resinous spots.

been defoliated one or more times, they may recover after the toxic agent has been leached down below the root zone. The initial stages of the leaf injury were not accompanied by visible evidence of root injury. An examination of many sections of boron-affected leaves failed to show effects upon the vascular system such as WARINGTON (6) found with the broad bean when the supply of boron was inadequate.

The effects of boron on lemon trees depend not only on the concentration of boron in the culture solution, but also upon the nature of the culture solution. When grown as sand cultures with one application of 9 liters of Hoagland's solution containing 50 p.p.m. of boron, lemon leaves showed a rapid destruction of the chlorophyll and a marginal burning. These effects were accompanied by a type of mottle, and the production of numerous dark brown or black slightly elevated resinous spots on the ventral surface of the leaf, which can even be seen to some extent on the dorsal surface, as shown in fig. 3. Similar spots may also be produced by a high concentration of sulphate. If we apply Hoagland's solution, enriched with additional calcium nitrate and 100–400 p.p.m. of boron, to Valencia orange trees, the leaves may become dry and curl tightly prior to their early abscission, without going through the preliminary stages of toxicity. These lethal concentrations produced effects upon the leaves that closely resembled permanent wilting.

Since a large part of the inorganic constituents of citrus leaves consists of calcium, it is of interest to study the effects of a nutrient solution containing boron but deficient in calcium. Such cultural treatment brought about a spotting of the leaves as a result of the burning of small areas between the veins (fig. 4), and also caused premature abscission. This indicates that the leaf symptoms resulting from toxic concentrations of boron are dependent to some extent at least on the concentration of other ions. BRENCHLEY and WARINGTON (1) found an association between the presence of boron and the absorption of calcium in certain plants.

The effect produced by known concentrations of boron in soil cultures was next studied. Valencia orange trees were grown in 10-gallon earthenware jars filled with soil, to which was added 2 liters of Hoagland's solution containing sufficient boron to give concentrations of 3, 6, 9, 12, and 15 p.p.m. of boron (in terms of dry soil). Control trees were also grown in soil which received the nutrient but no boron. All cultures then received 2 additional liters of Hoagland's solution. The drainage system of the crocks was controlled so that no solution escaped without being reapplied to the surface of the soil. After 11 months all of the trees in the boron-treated soil cultures showed typical boron injury. Only a few of the oldest leaves of the tree growing in soil containing 3 p.p.m. of boron showed effects, and these were so slight that, unless one were aware of the boron additions to the soil, they might easily remain unobserved. Had this been the case it might have been concluded that either the soil had the capacity to remove boron permanently from the field of root absorption, or that the supply of boron added to the soil was

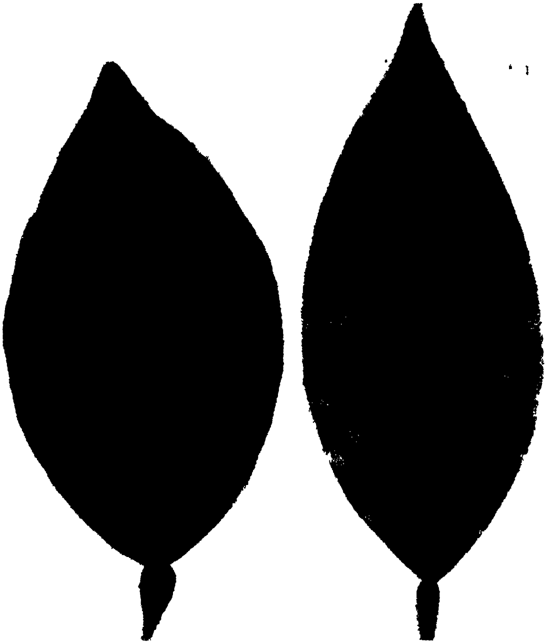

FIG. 4.—Toxic effects of boron on leaves of Valencia orange trees in sand cultures containing calcium carbonate as source of calcium; water-soluble calcium in cultures very low in relation to potassium.

insufficient to become an excess in the large tree top. The oldest leaves are the first to show an excess of boron.

The later cycles of growth of the trees, which were grown in soil containing 3 p.p.m. of boron, were normal in appearance. With a concentration of 6 p.p.m. or more of boron, the trees showed increased injury, as shown in fig. 5. The apical portion of the leaves was the first to show yellowing of the chlorophyll. As the leaves increased in age the yellow portion took on a bronzed appearance and developed tip burn. Gradually the chlorosis extended downward toward the basal end, the dark green color persisting longest near the larger veins. The effects are distinguishable in most cases from true mottle-leaf in that the terminal half of the leaf appeared to be af-

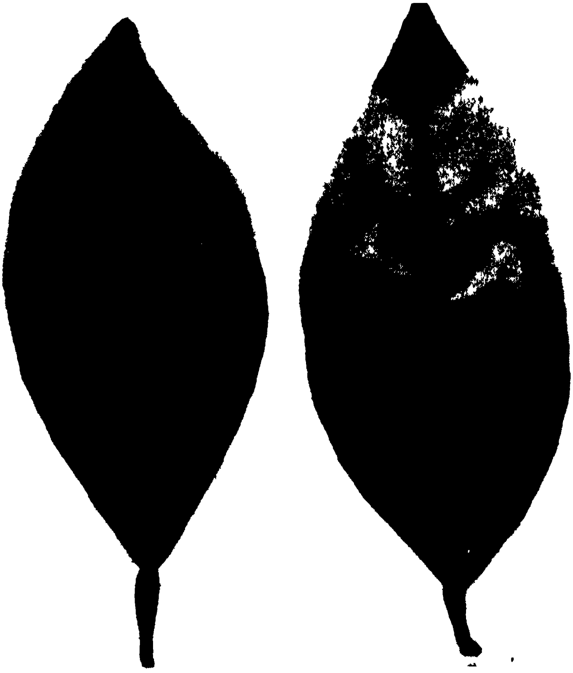

FIG. 5.—Toxic effects of boron on leaves of Valencia orange trees grown in soil cultures: left, ventral surface of leaf; right, dorsal surface of leaf; chlorosis progresses toward base of leaf blade, followed by burning of leaf tip and slightly bronzed coloration of chlorotic portion.

fected more than the basal half, and the tips and margins as a rule usually became brown.

The severity of the injury brought about by the addition of a given amount of boron decreases as the trees put on new cycles of growth. This is because the supply of boron is partially exhausted by the earlier growth. This fact indicates that there is very little if any migration of boron from the mature leaves out into the new growth; consequently the amount of boron required to produce injury is dependent on the size of the tree. Since in the state of nature many of the abscissed leaves are blown away, and soluble boron can be leached out of the soil, it is possible that the concentration of boron in the soil tends to be held in check by purely natural causes.

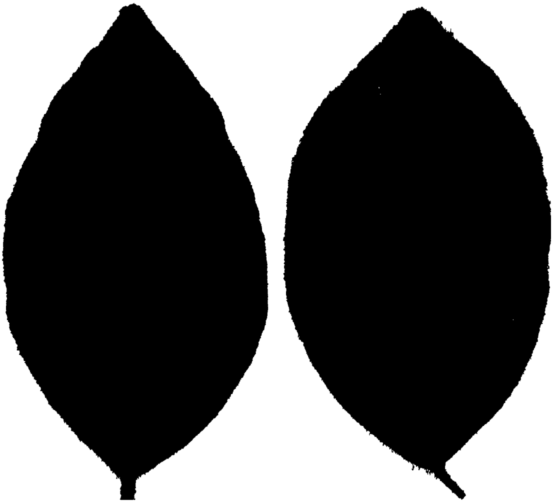

FIG. 6.—Effect of boron on mottling of leaves of Valencia orange trees in soil cultures that received sodium nitrate: left, slightly mottled leaf from cultures that received no boron; right, severely mottled leaf from cultures that received boron.

Under certain limited conditions the effect produced by boron may be not very different from that of true mottle. The Valencia orange leaf shown to the left in fig. 6 is typical of leaves grown in soil cultures receiving nitrate of soda. When small amounts of boron were added along with the nitrate of soda the mottle was intensified (fig. 6).

Lemon trees in soil cultures that received 5–10 p.p.m. of boron whenever irrigated, showed the boron effects seen in fig. 7. The leaves show marginal and tip burn, and the yellow areas between the veins also show burn. The resinous spots on the ventral surface of the leaf follow the progress of the chlorosis. Fig. 8 shows the effects of boron upon grapefruit leaves.

Lemon seedlings were grown in water cultures which received Hoagland's solution plus 7.5 p.p.m. of boron and varying amounts of iron sulphate. The ferric salt was used instead of the ferrous salt, in view of the statement (COMEY 3) that water may dissolve out all of the boric acid from ferrous borate but that ferric borate is insoluble in water. Fig. 9 shows the results obtained. All of the cultures received small amounts of ferric citrate from time to time.

The reduced growth at the higher concentrations of iron may possibly be related to the increased acidity of the iron solution, as well as to the excessive amounts of iron. There can be no doubt that the iron was effective in overcoming the toxic action of the boron. The growth obtained may have resulted either from the catalytic action of the iron or from the precipitation of the boron as insoluble ferric borate. BRENCHLEY and WARINGTON (1) have made the important observation that toxicity results from boron if iron is left out of the cultures.

The effect of other salts on the toxicity of boron was also studied. Among these aluminum sulphate, lead chloride, chromium nitrate, and zinc chloride may be mentioned. Of these salts only aluminum sulphate at concentrations up to 40 p.p.m. and lead chloride at concentrations from 65 to 170 p.p.m. appeared to give slightly better growth than the controls.

Boron toxicity was next studied with walnut seedlings grown in Hoagland's solution. Boric acid was added to some of the cultures in amounts ranging from 2.25 to 22.5 p.p.m. of boron. After 7 weeks the leaves of the plants which were grown in the solution containing 6.75 p.p.m. of boron showed curling and burning at the margin. At higher concentrations the leaves were curled and burned more severely, with a slight mottle occurring at the highest concentration.

In order to study the effect of concentration of boron on growth, several species of fruit trees were grown as sand cultures. The con-

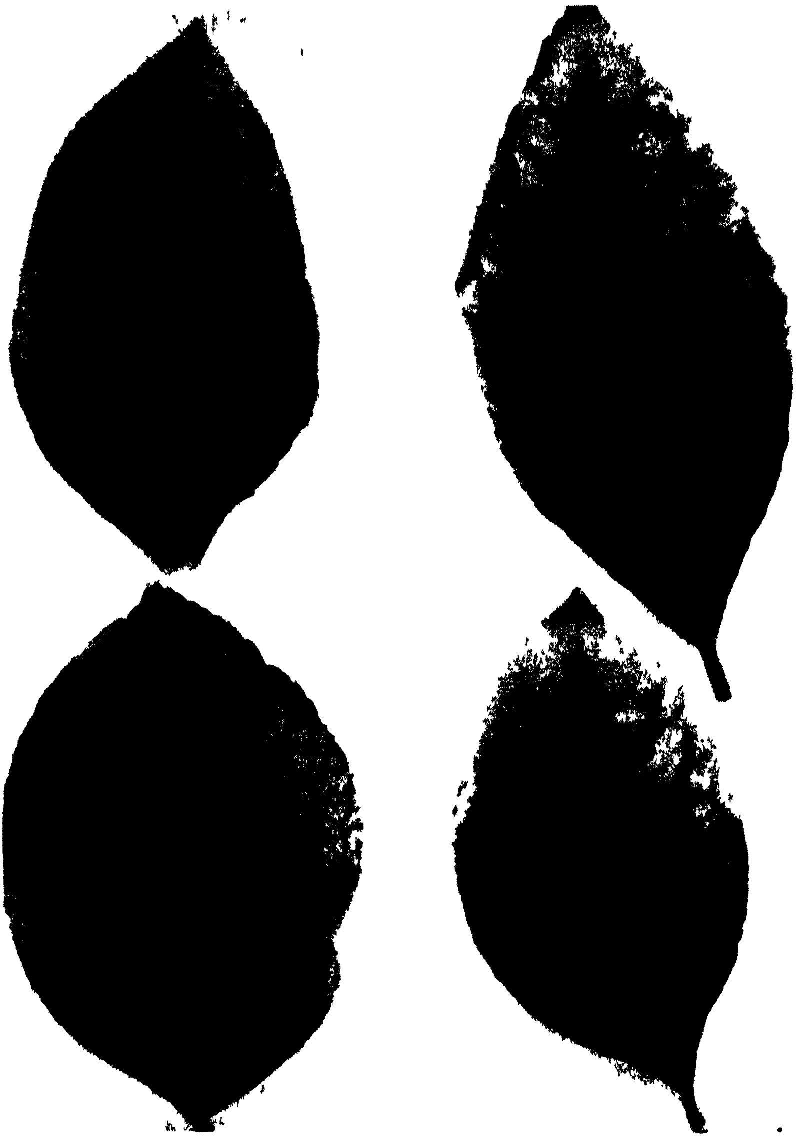

FIG. 7.—Toxic effects of boron on leaves of lemon trees in soil cultures: lower left, ventral surface of leaf showing resinous spots and marginal burning; lower right, leaf chlorotic with tip burn; upper right, further progress of chlorosis from leaf tip, tip burn, and marginal burning; upper left, burning of chlorotic areas and appearance of bronzed effect between veins.

tainers were 20 inches in diameter by 26 inches deep The containers were divided into three groups. The first group consisted of containers planted to Fuerte avocado (one), Eureka lemon (two), and Valencia orange (two), the second group consisted of containers planted to Stewart pecan (one), Pomelo (one), *Juglans regia* (one), Fuerte avocado (one), Elberta peach (two), and Eureka lemon (one), the third group consisted of containers planted to three to nine trees of each variety. The three groups of containers received half strength Hoagland's solution, with the addition of 1 p p m of boron to the first group, 2 p p m of boron to the second group, and no boron to the third group. Tap water was used in preparing the 50-gallon barrels of culture solution The solutions were thoroughly mixed and

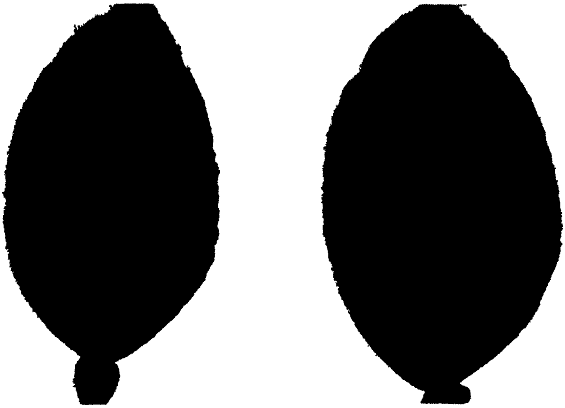

FIG 8 —Toxic effects of boron on leaves of grapefruit trees in sand culture

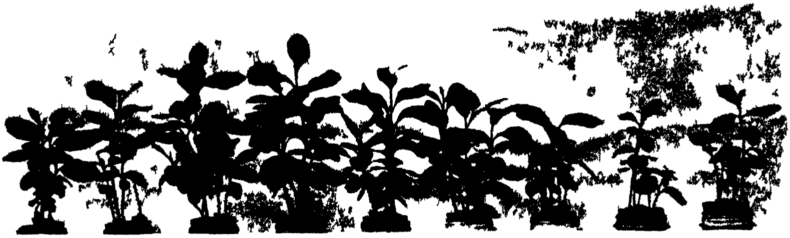

FIG 9 —Effects of ferric sulphate on growth of lemon seedlings in Hoagland's solution containing 7 5 p p m of boron, concentration of iron as ferric sulphate added left to right, 5, 10, 15, 25, 40, 65, 105, 0, 0 p p m

run in a fine continuous drip into the containers. The amounts of solution applied were considerably in excess of the transpiration requirements of the trees. In this way the desired concentration of boron in the sand cultures was maintained at a fairly constant level.

The experiment was begun in March, and in the following December leaf samples were taken for boron analysis. With concentrations of 1 or 2 p.p.m. of boron, the avocado leaves showed burned spots scattered throughout the entire leaf surface, but most conspicuous along the margin. These spots coalesced as the injury proceeded. When the culture solution contained 2 p.p.m. of boron the pecan leaves burned badly along the margin, and the new leaves were curled somewhat, which gave them a crinkled appearance. This condition of the avocado and pecan leaves is evident from fig. 10. Fig. 11 illustrates the effect of boron accumulation in peach leaves when the culture solution contains 2 p.p.m. of boron. The leaves burned severely along the entire margins and became wrinkled, later splitting as the drying out of the margins increased.

The leaves of the walnut tree in the culture that received 2 p.p.m. of boron showed burning in spots along the leaf margin. These coalesced, and finally the entire leaf margin became involved, as shown in fig. 12. At the same time many of the leaves became yellow between the veins, and subsequently they burned in these mottled areas as well as along the margin, as will be shown later in another connection. WARINGTON (5) found that boron injury with barley was characterized by a spotting of the leaves, while in the case of the broad bean there was a brown band along the margins. A spotting with burned areas on soy bean leaves has been produced with boron by COLLINGS (2).

In the sand cultures irrigated with a solution containing 2 p.p.m. of boron, the oldest grapefruit leaves showed boron injury only to a minor extent. The new growth was extremely vigorous, although toward maturity these leaves became somewhat pale in color and showed slight injury.

All of the mature leaves of the lemon tree treated with 2 p.p.m. of boron showed severe boron injury. The new leaves developed to full size but only the lowest ones have as yet shown any injury. The young leaves may make excellent growth for a time, only to be injured later. Since maturity of the leaves is greater the farther they occur from the shoot terminus, the injury due to boron is likewise greater in the same direction.

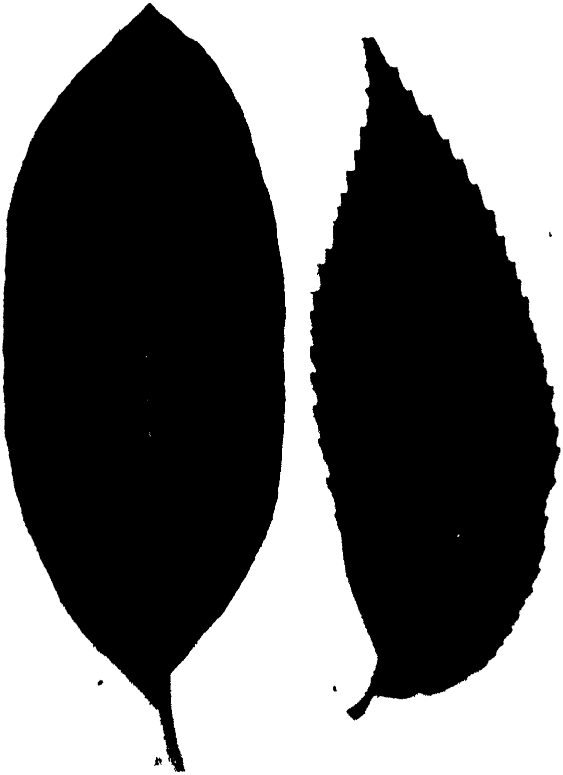

FIG. 10.—Toxic effects of boron on leaves of avocado and pecan trees in sand cultures: left, avocado leaf with dead spots scattered throughout and joining along edge to give appearance of marginal burning; right, pecan leaf with severe marginal burning.

The lemon and orange trees that received a culture solution containing 1 p.p.m. of boron showed no injurious effects. These trees grew most vigorously, and all of the growth was healthy in appearance. The orange trees made less growth than the lemon trees but the growth was normal in appearance. Leaf samples collected from these trees were analyzed for their boron content by MR. S. M.

BROWN. Table I gives the concentration of boron in the leaves of trees grown under controlled conditions, as compared with that of leaves from trees grown in the field.

The concentration of boron in the avocado leaves was not very high even in the affected leaves, although the amount present was relatively high when compared with that in normal leaves. The grapefruit leaves contained the largest amount of boron and still were not badly affected. Wide variation may exist in the boron content of leaves that do not show any symptoms. Lemon and orange leaves were found to contain over 100 p.p.m. of boron when only 1 p.p.m. was contained in their culture solution, and, notwithstanding this concentration of boron in the leaves, they still possessed a healthy appearance. When 1 p.p.m. of boron occurs in an irrigation water in the field, the concentration of boron in the soil solution is subject to considerable fluctuation as a result of such factors as evaporation of water from the soil, excessive irrigation, and frequency and amount of rain. As a consequence of these factors operating in the field, which have been described by KELLEY and BROWN (4), injurious concentrations of boron may be reached in the soil for the growth of citrus, a condition that was obviated in these controlled sand cultures.

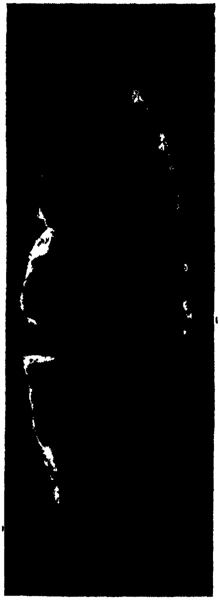

FIG. 11.—Toxic effects of boron upon leaves of peach trees in sand cultures.

Four-year-old walnut trees growing in the field at the Citrus Experiment Station were basined on September 27, 1926, and borax was applied to the soil. Two basins received 50 gm. each of c.p. powdered borax, two received 100 gm. each, two received 200 gm. each, and two received 400 gm. each. Adjacent trees served as controls. Only one application of borax was made.

On December 7, 1928, the trees in the basins that received 50 gm. of borax showed a slight trace of injury. Some of the leaves of the trees in the basins where 100 gm. of borax was applied became slightly chlorotic and mottled, but did not burn. At higher concentrations of borax the leaves mottled badly and burned between the veins. Many were badly crinkled and burned along the margins, as shown in fig. 13. When 200 and 400 gm. of borax were applied to the basins, the leaves of the second cycle of growth became full sized and were normal for a time; later they showed severe injury. The injury may not develop until after a considerable lapse of time, therefore, indicating a gradual accumulation of boron in the leaves. Boron determinations were made on samples of these leaves, the results of which are given in table II. The mottle and burning produced as a result of the borax applications resemble closely the condition of the leaves of many walnut trees in the field.

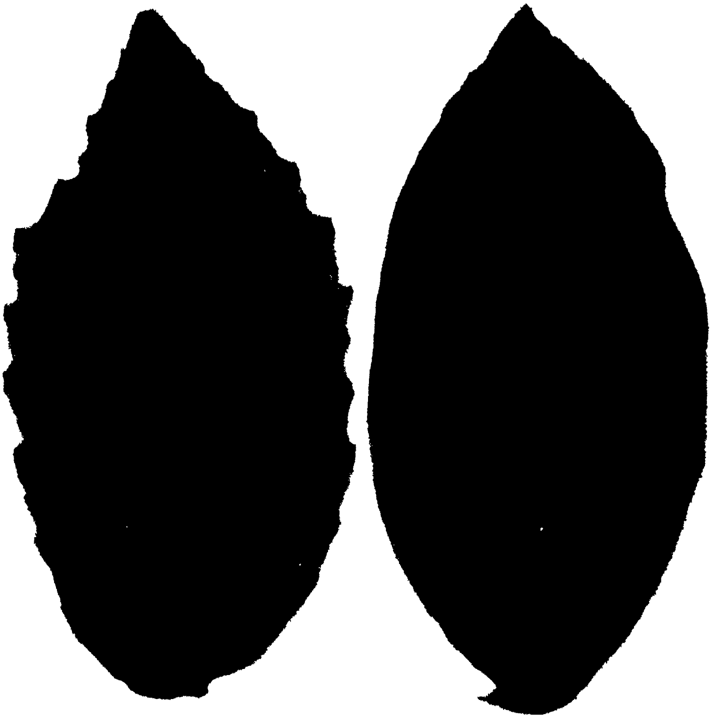

FIG. 12.—Toxic effects of boron on leaflets of walnut trees in sand culture: left, beginning stage of visible injury; right, advanced stage or marginal burning; mottle in both leaves screened out in making photograph.

The injurious effects of boron on the mineral nutrition of leaves are of considerable interest. Brenchley and Warington (1) have found evidence of a definite association between boron and the absorption or utilization of calcium. In the absence of boron in the nutrient solution the calcium was not fully utilized. In the present studies we are considering toxic concentrations rather than minimal requirements for boron, and therefore it is of interest to investigate the effects of toxic concentrations of boron on the calcium content of the leaves.

TABLE I

BORON CONTENT OF LEAVES OF TREES GROWN IN SAND CULTURES AS COMPARED WITH THAT OF LEAVES OF HEALTHY TREES IN THE FIELD

Tree	Location	Condition	Boron in culture solution (p.p.m.)	Boron in dry matter of leaves (p.p.m.)
Avocado......	Field, Box Springs Station	Healthy	....	20
Avocado.......	Sand culture	Affected	1	70
Avocado.......	Sand culture	Affected	2	82
Pecan.........	Field, Riverside	Healthy	....	24
Pecan.........	Sand culture	Affected	2	305
Walnut........	Sand culture	Affected	2	218
Grapefruit.....	Plot U, Riverside	Affected	....	34
Grapefruit.....	Sand culture	Slightly affected	2	480
Lemon........	Field, Box Springs Station	Healthy	....	29
Lemon........	Sand culture	Healthy	1	114
Lemon........	Sand culture	Affected	2	322
Lemon........	Sand culture	Full size healthy new leaves	2	54
Valencia.......	Field, Irvine	Healthy	....	24
Valencia.......	Sand culture	Healthy	1	120

The ash of orange and lemon leaves affected with boron was found to contain about 24 to 29 per cent of calcium and about 10 to 14 per cent of potassium. The ash of mature healthy orange and lemon leaves usually contains from 30 to 35 per cent of calcium and from 4 to 6 per cent of potassium. Citrus leaves injured by excessive boron therefore have a reduced content of calcium and an increased content of potassium. This type of composition corresponds to that of leaves that are mottled or immature. The excessive boron appears to poison the leaf tissues and to interfere with the absorption of calcium.

Samples of walnut leaves taken on September 26, 1927, showed the ash of the leaves from the trees where the largest application of borax was made, as containing 19.90 per cent of calcium and 12.52 per cent of potassium; while that of the leaves of control trees showed 24.71 per cent of calcium and 9.55 per cent of potassium. The ash composition of the leaves of these borax-treated walnut trees resembles that of mottled or immature walnut leaves. The ash of the water-soluble fraction of the dry matter of affected leaves was 27.74 per cent of the total ash, compared with 24.24 per cent for control leaves. The water-soluble calcium of the dry matter as a percentage of the total calcium was 4.44 per cent for affected leaves and 2.08 per cent for leaves of control trees.

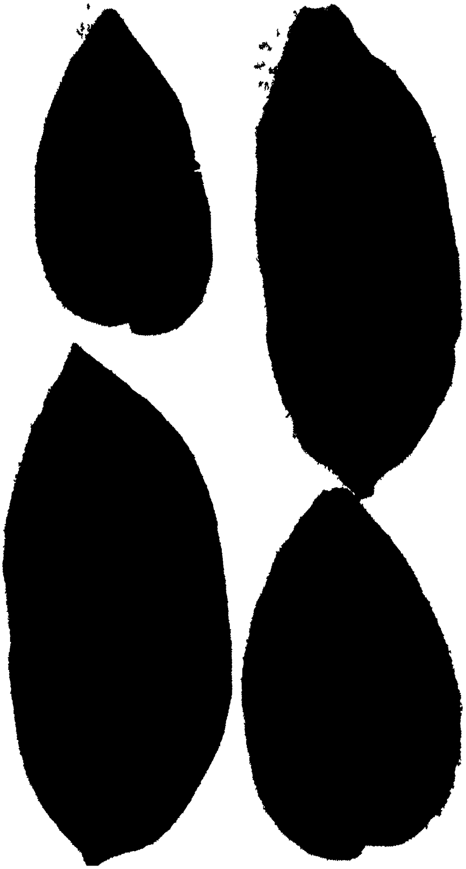

FIG 13—Toxic effects of single application of borax added to soil, on leaves of walnut trees in field; lower left leaf shows mottling, upper right leaf shows mottling to point of chlorosis and also marginal burning.

The soluble potassium was 86.84 per cent of the total potassium in the dry matter of the affected leaves while that in control leaves was 91.53 per cent. The total magnesium in the ash of affected leaves was 4.79 per cent while that in leaves of control trees was 6.25 per cent. The soluble magnesium expressed as a percentage of the ash of the soluble fraction was 6.33 per cent and as a percentage of the total magnesium was 36.61 per cent; while the values for control leaves were 13.64 and 52.83 per cent respectively. Leaf samples were taken from all of the trees again on December 7 and their analyses confirm these earlier results. An excess of boron therefore may disturb the mineral nutrition of leaves, and frequently so seriously that, as a result of the reduced calcium content, the leaves absciss prematurely.

TABLE II

BORON IN LEAVES OF WALNUT TREES THAT RECEIVED ONE APPLICATION OF BORAX

APPLICATION (GM.)	BORON IN DRY MATTER OF LEAVES (P.P.M.)
50	105
100	143
200	192
400	360

Summary

1. Boron is toxic to plants when present in relatively small concentrations.

2. Lemon seedlings are more sensitive to boron than orange seedlings. The new growth of seedlings affected with boron may be chlorotic.

3. By the continuous flow method of supplying the culture solution, the relationship was studied between the concentration of boron in the culture solution, the effect on the tree growth, and the concentration of boron in the leaves.

4. The effect of boron on citrus depends on the concentration of boron present in the nutrient solution and the concentration of other nutrient ions.

5. Additions of boron greatly intensified the mottling tendency of the Valencia orange trees when grown in soil treated with nitrate of soda.

6. Citrus and walnut leaves may become thin, mottled, chlorotic, and crinkled as a result of a toxic agent such as boron.

7. The addition of various amounts of ferric sulphate to cultures of lemon seedlings tended to overcome the toxicity of boron. So-

called insoluble borates in water cultures may furnish low concentrations of boron to the plant roots, but the continued absorption of these small amounts may be sufficient to produce toxicity. Several so-called insoluble borates were found to be distinctly toxic.

8. Unless badly defoliated repeatedly, trees injured by boron may recover if the toxic agent is leached out with water.

9. With toxic concentrations of boron, no changes in the vascular anatomy of the affected leaves were observed such as have been reported when boron was absent.

10. A single application of borax, ranging from 50 to 400 gm. per tree, to basined soil in which walnut trees were growing in the field produced a mottle and a leaf burn chiefly along the margins.

11. Citrus and walnut leaves affected with boron contain reduced amounts of calcium and increased amounts of potassium. The composition is typical of mottled and of immature leaves. The leaves, it is believed, are prevented from becoming mature in regard to ash composition as a consequence of the paralyzing action on the growth processes.

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CYTOLOGICAL STUDIES IN CYPERUS, ELEOCHARIS, DULICHIUM, AND ERIOPHORUM[1]

G CLAUDE HICKS

(WITH PLATES VIII, IX)

The presence of aneuploidy has been demonstrated in the Cyperaceae by two European investigators, in *Carex* by HEILBORN (11, 12, 13) and in *Scirpus* by HAKANNSON (10) In a previous paper, the presence of aneuploidy has been described in American species of *Scirpus* (14), and evidence adduced in favor of a hybrid hypothesis as an explanation of this condition. The present study deals with the chromosome conditions found in *Dulichium*, *Cyperus*, *Eriophorum*, and *Eleocharis*.

Materials and methods

The material was collected as it grew in the environs of metropolitan Boston and other points near the Atlantic seaboard The Gray Herbarium provided facilities for check work in the matter of plant determinations.

The inflorescences were collected on warm days and put immediately into Carnoy's fluid. The air was then drawn quickly from the tissues by means of an exhaust pump to insure rapid fixation. Material gathered in the early afternoon gave more satisfactory results on the whole. After being left in the fixative for 24 hours, the plants were washed in from two to four changes of 95 per cent alcohol.

It was generally found necessary to demineralize and bleach. A stock solution for such was made by adding crystals of sodium chlorate to strong hydrofluoric acid until a saturated solution was obtained. The material was passed into water and then put into wax or wax-coated bottles, containing a dilution of the fluid described, and left for only such time as was necessary for the required degree of softening and bleaching. Following this treatment the material was dehydrated and imbedded in nitrocellulose. A Jung-Thoma sliding microtome was used for cutting sections. Both longitudinal and transverse sections were cut 10 and 5 μ thick. It was demonstrated that transverse sections were the best for details.

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[1] Contributions from the Laboratories of Plant Morphology, Harvard University.

Haidenhain's iron-haematoxylin was used for staining, and gave satisfactory results. A Bausch and Lomb microscope equipped with a 140° Abbé condenser was used. Since strong light and resolution were very important factors, a 3 mm. 140° Bausch and Lomb apochromatic lens, together with a no. 15 periplan compensating ocular, provided an excellent combination of equipment. The drawings were made with the aid of a camera lucida and then enlarged three times.

Counts of the chromosomes were made mostly from the heterotypic metaphase plates, and where possible use was made of diakinetic and homeotypic stages.

The chromosomes in *Cyperus* are rather small, and it is difficult to know the exact conditions of affinity from a study of diakinesis. The other genera, particularly *Eleocharis*, display almost diagrammatic conditions. It has seemed best not to group the chromosomes into classes as no consistent results are obtained upon the whole. Haploid counts of the chromosomes are recorded.

CYPERUS

Cyperus dentatus Torr.—Material from Long Pond, Natick, Massachusetts, shows 17 chromosomes (fig. 3), one of which is conspicuously larger than the others. The chromosomes behaved normally in all the divisions forming the regular four nuclei, three of which aborted in the usual manner. The spikelets of this species are often abortive and are changed into leafy tufts, even though the pollen is rather well formed up to the time of shedding.

Cyperus sp.—This species, collected by JEFFREY in New Zealand, could not be identified as the material was not mature enough. The spikelets are rather broad, and there is one anther only to a flower. According to CHEESEMAN (6) it may be *C. tenellus* L. It would appear that all the chromosomes are bivalents. The metaphase plates (fig. 4) always show 21 chromosomes, two of which are conspicuously larger than the rest, 7 are smaller, and 12 are of medium size. The large chromosomes not infrequently show a twofold nature as if they were quadrivalents, and but very little material staining darkly is found in the cytoplasm.

Cyperus erythrorhizos Muhl.—Plants were collected at Winter Pond, Winchester, Massachusetts. The chromosomes are disorderly in arranging themselves at the metaphase plate (fig. 5). The 48 elements seen are much smaller than those found in diakinesis. This difference may be due to clumping or may be due to weakened affinity. Interkinesis is apparently of short duration. The surviving nucleus divides very promptly after the abortive nuclei have begun their degeneration.

Cyperus esculentus L.—Material found at Jamaica Pond, Boston, proved suitable for study. Fig. 6 shows a heterotypic plate. While at times there is a grouping of the chromosomes so that accurate counting cannot be made, there are also good clear plates which possess 54 chromosomes of various sizes. All stages of division are regular.

Cyperus filiculmis Vahl. var. *macilentus* Fern.—Collections of this species that proved of value were made at South Sudbury station and at Sharon, Massachusetts. The anther sacs are extremely small, and as the pollen mother cells are not more than 12 cells in height, much material had to be sectioned to provide a basis for accurate results.

Diakinesis stages show larger chromosomal elements than the metaphase plates. It is in the movement of the chromosomes from the metaphase plate that one comes upon irregularities not unlike those found in the irregular species of *Scirpus americanus* (14) found at Hyannis, Massachusetts. Fig. 8 shows this condition. Fig. 7 shows a metaphase plate. There are 73 chromosomes which are exceedingly difficult to delimit. This number cannot be considered as final, for not enough clear plates have been available. The homeotypic divisions seem to be regular, and cell plates are found in both divisions.

Cyperus strigosus L. var. *compositus* Britton and *C. ferax* Rich.—These have been found to have very large chromosome numbers, but the non-availability of good clear figures, as noted in *C. filiculmis* var. *macilentus*, has only provided inconsistent and unreliable results. The former is open to the suspicion that it is heterozygous material.

ERIOPHORUM

Eriophorum virginicum L.—Fig. 2 shows good clear plates from plants collected at Tewkesbury, Massachusetts. Twenty-nine chromosomes have been found to be present: 2 larger, 8 smaller, and 19 of medium size. It will be seen that the chromosomes are split in preparation for the homeotypic. All stages have been found to be regular.

Eriophorum gracile Roth. and *Eriophorum tenellum* Nutt.—These showed only mature pollen.

DULICHIUM

Dulichium arundinaceum (L.) Britton.—Material of this species was gathered at Wakefield, West Manchester, and Bedford, Massachusetts. Of all the species in the present connection this was the most difficult to differentiate, as the cytoplasm possessed a very "muddy" appearance. No stages of diakinesis have been studied satisfactorily. The anthers are exceedingly long, and starting at the top of the anther sac with the heterotypic metaphase, we find all transitions, to the late anaphase at the bottom. Both divisions are regular, and pollen formation is normal. In all cases, 16 chromosomes (fig. 1) have been found.

ELEOCHARIS

Eleocharis obtusa (Willd.) Schultes.—This is recorded as being "very variable in size and habit" (24). Several collections were made at Moncton, N.B., Canada, one at Glenwood, Massachusetts, and another was kindly sent by Dr. A. E. LONGLEY from Washington, D.C. This species may be most easily confused in the field with *E. ovata* (Roth.) R. & S. Accordingly, material was collected only from clumps in which plants showed the "tam o' shanter" cap on the achenes. This is possible because both flowering and mature material are found in the same clump.

The haploid number is shown to be 5 large and equal chromosomes, which is low. The behavior in all regions is practically the same. Fig. 9 shows a late diakinesis, 4 of the chromosomes having lost the bivalent configuration which has been retained by the fifth.

This might also be interpreted as the cause of the slight lagging sometimes observed.

Dark material is seen in the cytoplasm. The heterotypic metaphase is quite orderly. The spindle fibers end in a point, a rather exceptional condition.

The plants from Washington were the only material to show any irregularity in the heterotypic metaphase. These were very tall. In the homeotypic anaphase, however, there were in addition to the fairly regular figures, those in which the chromatin was strung out along the fibers, and it was impossible to delimit the chromosomes. Four nuclei were always seen upon the termination of the divisions.

The pollen at the time of shedding is very much shrunken, although before that time it appears quite normal.

Eleocharis sp.—An unidentified species was collected by JEFFREY in New Zealand, and it is interesting from the standpoint of chromosome number. In all stages (diakinesis etc.) there are present 10 chromosomes. The divisions are regular. One large chromosome is conspicuous from the others (fig. 10).

Eleocharis tuberculosa (Michx.) R. & S.—A single collection of this plant was made in a wet region in the Concord River meadows at Bedford, Massachusetts, and the preservation of the material was very poor. The metaphase plates were for the most part hopelessly clumped together, and a suitable study of diakinesis was impossible because of cytomixis.

In several anther sacs, clear plates of the heterotypic metaphase possessed 15 chromosomes (fig. 11), which may be divided into classes of average size, 7 larger and 8 smaller. While the chromosomes could not be checked in their behavior, from the gross appearance meiosis was quite normal and 4 nuclei only were formed. The pollen was somewhat shrunken at the time of shedding, but there were always considerable protoplasmic contents in the cells.

Eleocharis capitata (L.) R. Br.—The nomenclature of this species has been revised by BLAKE (4): it is the *E. tenuis* (Willd.) Schultes of GRAY's manual. Material from Chestnut Hill, Massachusetts, and from Wolfville, N.S., Canada, which Professor R. H. WETMORE kindly collected for me, showed similar conditions.

Size differences are seen in the polar views of the metaphase plates (fig. 12). In diakinesis there is a quadrivalent arrangement of two of the chromosomes. This affinity at other times forms a large mass which appears to be made up of more than four elements. This quadrivalent formation must be only transitory, however, since it has never appeared in the metaphase plates. The nucleolus possesses a scalloped appearance and it is soon to disappear. Its degeneration is marked first by small vacuoles or areas that do not take the haematoxylin stain. These vacuoles soon coalesce, and only the border retains the normal dark blue color. Later on the whole nucleolus fades and is no longer seen.

The size differences of the chromosomes are seen to be of two average classes, 4 larger and 15 smaller. In the upper left part of the pollen mother cell is a black mass. This is one of the larger chromosomes passing over in cytomixis from the metaphase plate of an adjoining mother cell. The heterotypic anaphase is normal; the state of interkinesis is of some duration, and in this stage nucleoli are very seldom apparent. In the homeotypic anaphase the size differences are again seen. The abortion of the degenerating nuclei is entirely normal.

Eleocharis palustris (L.) R. & S.—In GRAY'S manual, seventh edition, this species is cited as "very common and very variable either in water, where it is rather stout and tall, or in wet grassy grounds, where it is slender and lower."

A comprehensive study of this species was made, and the results show from a morphological point of view that the species is really a group. Counts were made in diakinesis and in the heterotypic and homeotypic metaphases. It was demonstrated clearly that only the early stages of diakinesis were of value; then the pairing chromosomes from Y's, O's, X's, etc. Soon each of the bivalent masses gives way to a rounded mass, and the later stages are no longer of value and much more difficult to study than the metaphase plates. The slender and lower types of the species mentioned showed the haploid number to be 8. Fig. 13 shows the condition in plants growing along the wet shores of Lake Waban, Wellesley, Massachusetts. While it is to be remembered that chromosome size appears to be largely a matter of averages, it is obvious that there are size differences in the chromosomes: 2 more massive, 1 medium, and 5 smaller. The divisions are quite regular, excepting that soon after diakinesis dark material is seen in the cytoplasm. It will be noted that a constriction occurs in the chromosomes of the heterotypic metaphase plates (figs. 13, 15). This appearance has been noticed in wheat and other material, and described as a preparation for the homeotypic divisions. The condition here persisted for a surprisingly long time in interkinesis and even in the aborting nuclei. This same split condition occurs in the divisions of the embryo sac mother cell.

In members of the species collected at the head of Spot Pond and at a pond locally called Frog Pond in Chestnut Hill, however, there appeared occasionally in the metaphase plates 9 chromosomes in place of the usual 8. Fig. 14 shows such a condition. This appearance is probably due to the failure of the bivalents to pair. In anther sacs with this abnormal number, the chromosomes at times showed a lack of regularity. Fig. 16 shows lagging of the chromosomes in the anaphase. At the left it would appear that non-disjunction might take place. While the twofold nature of the chromosomes is often confusing, at the end of the heterotypic anaphase several clear figures have shown more than the normal elements. Counts of the young pollen grains, however, have never shown more than 8 chromosomes.

More interesting still are the variants of the stout plants growing in the water at Heard's Pond, Wayland, Massachusetts. There diakinesis often shows the bivalent pairs rather well, and conspicuous among the contents is always a "pair" which is not to be taken for a tetrad. Its behavior shows its elements to be quadrivalent in nature, and the halves are found not only lying side by side but also end to end. The metaphase plate (fig. 15) shows 18 chromosomes, 5 larger and 13 smaller of average sizes. Of the 5, two usually form the pair just mentioned, and a further pair may be formed by two of the remaining elements. Fig. 17 shows a profile of the heterotypic metaphase, and foremost among the elements there is a large quadrivalent chromosome. It may be said in passing that such structures as these have been found in tetraploid plants.

The heterotypic anaphase has never shown any irregularities. In the case of the homeotypic division (fig. 18) we find a distinct lagging, however, and conditions quite similar to those in fig. 16.

It would seem at times that the chromosomes failed to separate. Countings of the chromosomes in the young pollen grains, at the present time, seem to indicate that such happens. The pollen grains and pollen mother cells are much larger in the stouter form.

Eleocharis palustris (L.) R. & S. var. *glaucescens* (Willd.) Gray.—Only one of the collections proved to be of this variety. This was made at one of the smaller ponds near Fresh Pond, Cambridge. The homeotypic anaphase is regular, as were all the stages seen. In no case did there appear more than 4 nuclei in a pollen mother cell, contrary to the report of WILLE (33) in *Eleocharis palustris*, where 3 smaller and 2 larger ones were seen. In the larger forms growing at Wayland and Bedford, apparently every little seed was set, although the pollen was uniformly good before the time of shedding.

Eleocharis acicularis (L.) R. & S.—Members of this extremely small sedge were gathered at Heard's Pond, Wayland, Massachusetts, and at a small pond by the side of the road that runs past the Belmont Country Club.

The inflorescences suitable for cytological study are about 2 mm. long by 1 mm. broad, so that the anthers are extremely small and a great amount of material has to be cut to obtain the right stages. The material from Belmont showed the clearest results. The late prophase reveals, in the majority of cases, 28 chromosomes arranged in varying affinity. For the most part they are found linked in threes, but there are also to be found fours, twos, and ones. A like situation occurs in the metaphase plates (figs. 19, 20, 21). At times the chromosomes also appear as if in a serial arrangement, as described in *Carex aquatilis* by STOUT (28).

The number of chromosomes found in an inspection of the metaphase plates is a varying and puzzling one. In fig. 19 there are 26 of varying sizes, a smaller chromosome (chromomere?) being conspicuous. In figs. 20 and 21 are 29 chromosomes, but the sizes are different. These conditions are particularly puzzling as they may be found in anther sacs of the same plant. The chromosome numbers in these plates range from 25 to 29. Some of the differences may be explained in part by the fact that a group of the chromosomes may become attached longitudinally along the fibers of the spindle. Another feature is the lack of synchronization of chromosome movement, for in one and the same anther sac there may be observed the heterotypic metaphase, heterotypic anaphase, and homeotypic metaphase.

The early anaphase of the heterotypic is always extremely irregular (fig. 22), with tangled figures, the elements of which are extremely difficult to delimit. The lagging of the chromosomes is observed in the late anaphase, but there have been seen no cases of chromosomes being left out in the cytoplasm.

Figs. 23 and 24 show the conditions found in the homeotypic metaphase plates. Counts vary, 18, 19, etc. Some of the trivalents have become oriented longitudinally on the spindle (fig. 24), and are disjoined in this division. Never has the same number of chromosomes been recorded as in the heterotypic plates.

In the anaphase of the homeotypic division there is extreme irregularity in the movement of the chromosomes. The elements of trivalent chromosomes go two to one pole and the remaining part to the other pole. At maturity there are often to be found anther sacs of shriveled pollen. Counts in the surviving tetrads have been made and are 15, 16, and 17. To establish the exact number, clear somatic plates unavailable at the present time will have to be examined. The material provided exceptionally clear and diagrammatic stages in the development of the embryo sac mother cell.

Discussion

When plants and animals are characterized by polymorphy, the behavior of the chromosomes in meiosis, and the quality of the reproductive bodies accompanying such diversity of form, are apparently characteristic. The evidence that polymorphy is very often seemingly due to a heterozygotic condition is now abundant and well known. It is conceivable that hybrid plants may not be abnormal in their reproductive behavior. If two plants closely enough related to one another are crossed, disturbances may not be more obvious than in pure strains. This has been demonstrated in known hybrids. It is also not a foregone conclusion that because a plant is of heterozygous origin it will be polymorphic. Constant hybrid blends are numerous and need no recital.

It will be the aim of this discussion to point out just how far we may interpret the abnormal phenomena here, in the light of hybridization.

The natural hybrid *Drosera obovata* investigated by ROSENBERG (25, 26) presents a clear case of meiotic irregularities caused by univalent chromosomes. This species has as parents *D. longifolia* (20×) and *D. rotundifolia* (10×); consequently there are present 10 bivalents plus 10 univalents. The univalents are frequently left out in the cytoplasm, where they form small nuclei, and in the second division may develop small spindles. Disorganization of the pollen grains follows.

WODSEDALEK (35) has investigated the causes of sterility in the mule. There are no cases of its being interfertile. The mitoses found in the spermatogonial (somatic) cells are quite normal, but in the late prophase of the primary spermatocyte, the pairing of the chromosomes is never complete and is very inconsistent, as seen in the number of the chromosomes present. These range from 34 to 49, as compared with the 59 found in the spermatogonial cells.

The roses have long been distinguished by their polymorphy, and the cause was unknown or only suspected. The work of TÄCKHOLM (30) and of BLACKBURN and HARRISON (2) has demonstrated the concomitant cytological conditions. TÄCKHOLM divides this tremendous genus into three great classes: (1) those characterized by the occurrence of only paired chromosomes; (2) the very polymorphic Canina section in which bivalents and univalents are found usually in multiples of 7; and (3) aneuploid forms in which bivalents and univalents do not form multiples of 7. The first group contains diploid, tetraploid, hexaploid, and octoploid species and hybrids. The hybrids include many forms that are regular in the reproductive divisions and in spore formation. The second group, because they are always characterized by the presence of bivalents and of univalents, are analogous to the *Drosera* scheme of hybrids, and are considered by TÄCKHOLM to be F_1 generations. They are known for their widespread sterility, and have maintained themselves by apomixis. The Canina group by their crossing have given rise to the third group, which has irregular chromosome numbers because of irregularities in the chromosome number and in distribution. That aneuploid or dysploid numbers are brought about by the agency of hybridism is a very important and significant fact to consider in connection with the explanation of the origin of aneuploidy in other plants, for the roses are not an exception in this regard.

The presence of 9 chromosomes in some cells of *Eleocharis palustris* recalls the behavior described by ROSENBERG (27) in *Crepis*, and he has used the occurrence of irregularities in chromosome distribution to explain the increase in chromosome number of recently originated *Crepis* forms. He believes that the *Crepis* species with three chromosomes have arisen from four chromosome forms by an occasional failing of two chromosomes in the pollen mother cells and embryo sac mother cell to pair in diakinesis, with the result that there are three paired and two unpaired chromosomes. When the chromosome distribution is irregular, some daughter nuclei receive five and others receive three chromosomes If like gametes met like, the result would be the origin of three and five chromosome species of *Crepis*.

The work of WINGE (29) on crossing as the cause of polyploidy has been well established. In *Rubus* and *Crataegus*, LONGLEY (19, 20) has investigated the causes of polymorphy, and has come to the conclusion that the species have been multiplied by hybridization In *Crataegus* he finds three classes: (1) diploid species in which pollen formation is normal; (2) triploid and tetraploid species that show irregularities in their chromosome distribution and are accompanied by polycary and polyspory, and (3) triploid and tetraploid species that are unable to form pollen grains and are sterile because they are the products of distant crosses. In *Rubus* he finds two classes: (1) diploid species in which meiosis and pollen formation are normal, and (2) polyploid species which are triploid, tetraploid, pentaploid, and octoploid These polyploid forms are seemingly hybrid because characterized by irregularities in chromosome distribution which leads to polycary and polyspory. The fact that species have been modified by hybridization in their natural habitats is of fundamental importance in the consideration of other groups.

HOLMGREN (15) investigated *Erigeron micranthus*. Usually there are 13 pairs of chromosomes; frequently there are only 11 or 12, and the univalents which are formed as a result of the weakened affinity behave as supernumeraries and are distributed in varying ways to the daughter nuclei. This is an indication that this species was derived from parents which differed in their chromosome constitution but very little. TISCHLER (31) regards these conditions in *Crepis* (reported by ROSENBERG) as being due to a like cause.

The Boston fern has been the subject of much study regarding its variations, which have been regarded as mutations. This fern is marked by its sterility and its great vegetative vigor. The apparent cause of polymorphism in this species has been described by JEFFREY and ROSCOE (18). The reduction divisions are quite abnormal, and there are conditions of lagging univalent chromosomes such as are found in known hybrids. Sporangia are shrunken and the development of the spores is generally entirely abortive. Accordingly this so-called mutant fern is to be regarded as a hybrid. Hybrid species of ferns with a high degree of probability often originate in greenhouses as well as in nature.

Many of the species regarded as examples of mutation have been shown to be of heterozygous origin. More recently *Drosophila melanogaster* has been shown to be abnormal in its divisions, both as to the presence of more than the expected number of chromosomes, and as to the absence of an equatorial plate typical in the metaphase, and the extrusion of chromosomes into the cytoplasm. These peculiarities may be considered to indicate the heterozygous origin of this much investigated insect.

The striking sets of homologous chromosomes described here in the small sedge *Eleocharis acicularis* have previously been reported in both plants and animals.

OSAWA (22) found trivalents occurring in triploid races of *Morus*, and by hybridizing several diploid species and races he was able to duplicate the trivalent condition. BELLING (1) has investigated triploid races of *Canna*. The chromosomes formed triads, and in the heterotypic split, and passed, two to one pole and one to the other. About half the pollen grains were devoid of cytoplasm. In regard to this condition in *Canna*, TISCHLER (31) states "zweifellos als Bastarde."

The recent investigations of maize have revealed the presence of trivalent chromosomes. LONGLEY (21) reports them in a cross between *Zea mays* and *Euchlaena perennis*. RANDOLPH and Mc-

CLINTOCK (23) still more recently have reported trivalents in triploid races of *Zea mays* which apparently were crosses between a dilute sun red race and a heterozygous tunicate tassel.

The question as to the order of succession of the reduction and the equation divisions has been the subject of considerable discussion. WILSON (34) states:

> This long disputed question must obviously rest upon our means of identification of the reduction divisions and . . . . such identification can only be made with complete certainty in cases where the synaptic mates are visible, distinguished by differences of form, size, structure, or mode of attachment.

A comparison of the polar views of the heterotypic metaphase plates, together with profile views of the same and the conditions found in the corresponding phases of the homeotypic, apparently leads to the conclusion that the reduction and the equation divisions are mixed in *Eleocharis acicularis*. The case of *E. palustris* is rather remarkable because there has not only been a doubling of the number of chromosomes, but also the addition of odd units. The extra individuals may possibly be explained by the evidence of non-disjunction, or by the weakened affinity seen in the few cases in the material from Spot Pond and Chestnut Hill, where 9 chromosomes were found in the metaphase plates. The material used in this investigation, however, may not be entirely representative of the species. As the writer has studied material from only seven stations, the exact method of duplication may reveal itself in further investigations.

A notable instance of a tetraploid form arising from diploid stock is found in the case of *Primula kewensis* (8). This plant arose as a hybrid in 1899 between *Primula florabunda* and *P. verticillata* at Kew Gardens. A peculiarity analogous to the quadrivalents seen in the large forms of *Eleocharis palustris* was found in the temporary linking of two bivalent chromosomes in the heterotypic division, which association persisted until the beginning of the anaphase. Like conditions have been reported in *Rosa wilsoni* by BLACKBURN and HARRISON (3). The parents of this species are *R. pimpinellifolia* and some *Tomentosa microgene*. CLAUSEN and GOODSPEED (7) found tetraploid forms in *Nicotiana* hybrids resulting from a cross of *N. glutinosa* (12×) × *N. tabacum* (24×). They claim it to be an experimental verification of WINGE's hypothesis that higher chromosome numbers are derived from lower ones by crossing. TSCHERMAK and BLEIER (32), in their account of *Aegilops* and *Triticum* hybrids, believe also with WINGE that polyploidy has come about through crossing. The species *T. ovata*, *T. dicoccoides*, and *T. durum* possess 14 x chromosomes, but in fertile crosses there is a doubling and consequently there are 28.

Similar conditions have been reported in *Digitalis* crosses by HASSE-BESSELL (9), and in sugar cane by BREMER (5).

Conclusions

The cytological conditions described in the genera under discussion are remarkably correlated to the taxonomic variability recorded for the different species. In *Eleocharis* it has been shown how higher numbers have probably arisen and also how they have become modified in *E. palustris*. In this species in the stouter and taller races the number of chromosomes has become doubled and been further modified by some means; perhaps by non-disjunction of the chromosomes. This doubling has most probably come about through hybridization, and as a consequence further modifications have arisen.

The plants of *Eleocharis acicularis* investigated also present conditions that may be interpreted as indicating hybrid derivation. The peculiarities in the union of homologous chromosomes, unstable conditions in pairing, and lagging of chromosomes seem, in the light of known conditions of the origin of such structures, to mark the heterozygous nature of this plant. The weak pairing of the chromosomes seen in *E. obtusa* resulting in irregularity, together with the known variability of this plant, indicate that it may have suffered hybrid contamination.

These conditions, in a genus that is known upon the whole to be comparatively stable taxonomically, provide significant indication of the mode of origin of aneuploidy in other groups, namely, through crossing. *Cyperus* further presents evidence that falls in line with other genera investigated. This is illustrated by *Cyperus filiculmis* var. *macilentus* and also probably by the variety of *C. strigosus* here described. In view of the cytological evidence presented, it is to be concluded that *Eleocharis* and *Cyperus* are being modified by crossing, and it is highly probable that the same situation has also prevailed in the past.

An interesting question arises in regard to the conclusions reached by HEILBORN in *Carex*. He came to the conclusion that the aneuploidy found in this genus is the result of mutation and has no obvious connection with previous hybridism. It seems unlikely, in view of the conditions here recorded, that this point of view can be maintained, at any rate for the genera studied in this investigation.

Summary

1. *Eleocharis*, so far as studied, has the following aneuploid chromosome numbers: 5, 8, 8–9, 15, 18, 19, and 26–29.
2. *Cyperus*, so far as studied, has the following aneuploid chromosome numbers: 17, 21, 48, 54, 73, and variable.
3. *Dulichium arundinaceum* has 15 chromosomes.
4. *Eriophorum virginicum* has 29 chromosomes.
5. Conditions similar to those found in known hybrids have been discovered in *Eleocharis* and *Cyperus*.
6. Cytological indication of hybrid origin is clearly correlated with taxonomic variability and polymorphy.
7. Hybridism is offered as a probable explanation of the aneuploidy found in the Cyperaceae.

The writer wishes to record his indebtedness to Professor E. C. JEFFREY, at whose suggestion this research was undertaken and under whose supervision it was carried out. Appreciation is also expressed for Professor M. L. FERNALD'S valued assistance with plant determinations.

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EXPLANATION OF PLATES VIII, IX

PLATE VIII

FIG. 1.—*Dulichium arundinaceum*, heterotypic metaphase.

FIG. 2.—*Eriophorum virginicum*, heterotypic metaphase.

FIG. 3.—*Cyperus dentatus*, heterotypic metaphase.

FIG. 4.—*Cyperus* sp. (New Zealand), heterotypic metaphase.

FIG. 5.—*C. erythrorhizos*, heterotypic metaphase.

FIG. 6.—*C. esculentus*, heterotypic metaphase.

FIG. 7.—*C. filiculmis* var. *macilentus*, heterotypic metaphase.

FIG. 8.—Same, heterotypic anaphase.

FIG. 9.—*Eleocharis obtusa*, diakinesis.

FIG. 10.—*Eleocharis* sp. (New Zealand), heterotypic metaphase.

FIG. 11.—*E. tuberculosa*, heterotypic metaphase.

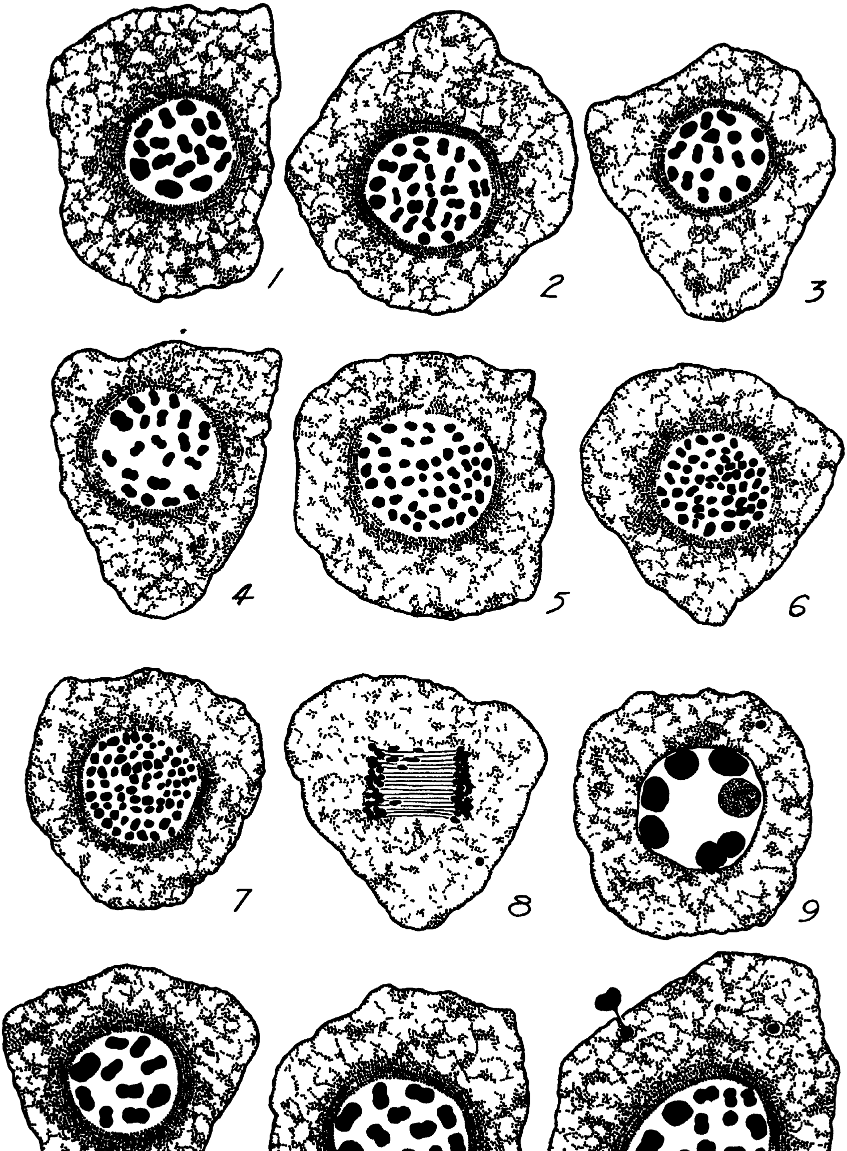
FIG. 12.—*E. capitata*, heterotypic metaphase.

PLATE IX

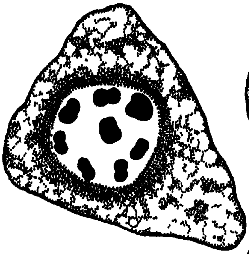
Eleocharis palustris

FIG. 13[illegible]terotypic metaphase showing 8 chromosomes.

FIG. 14.—Heterotypic metaphase showing 9 chromosomes.



HICKS on CYPERACEAE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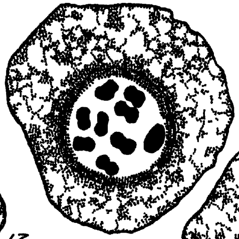
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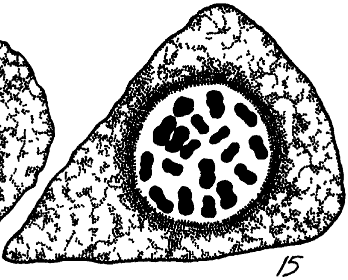
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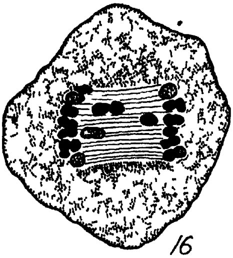
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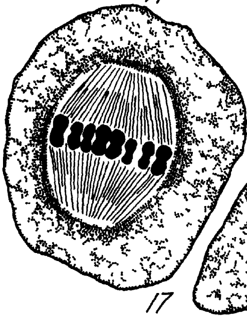
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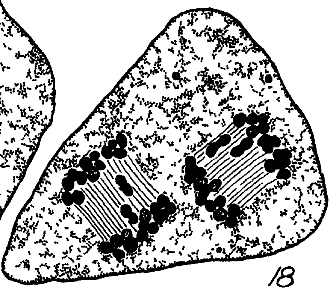
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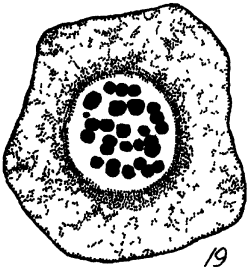
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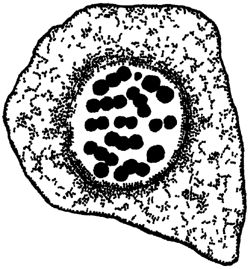
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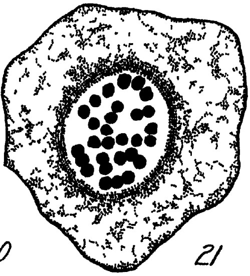
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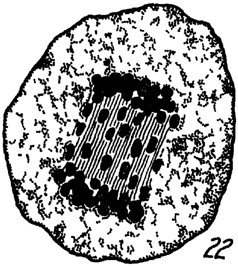
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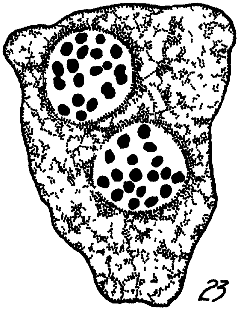
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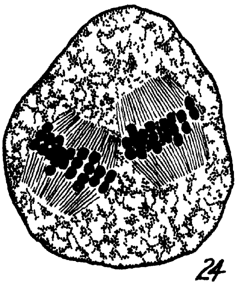
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HICKS on CYPERACEAE

FIG. 15.—Taller and stouter forms, showing 18 chromosomes.

FIG. 16.—Heterotypic anaphase.

FIG. 17.—Taller and stouter forms, showing side view of heterotypic metaphase.

FIG. 18.—Homeotypic anaphase.

Eleocharis acicularis

FIG. 19.—Heterotypic metaphase showing 25 chromosomes.

FIG. 20.—Twenty-nine chromosomes.

FIG. 21.—Twenty-nine chromosomes.

FIG. 22.—Heterotypic anaphase.

FIG. 23.—Homeotypic metaphase, polar view.

FIG. 24.—Homeotypic metaphase, side view.

MORPHOLOGY OF SPOROPHYTE OF MARCHANTIA DOMINGENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 394

EMMA N ANDERSEN

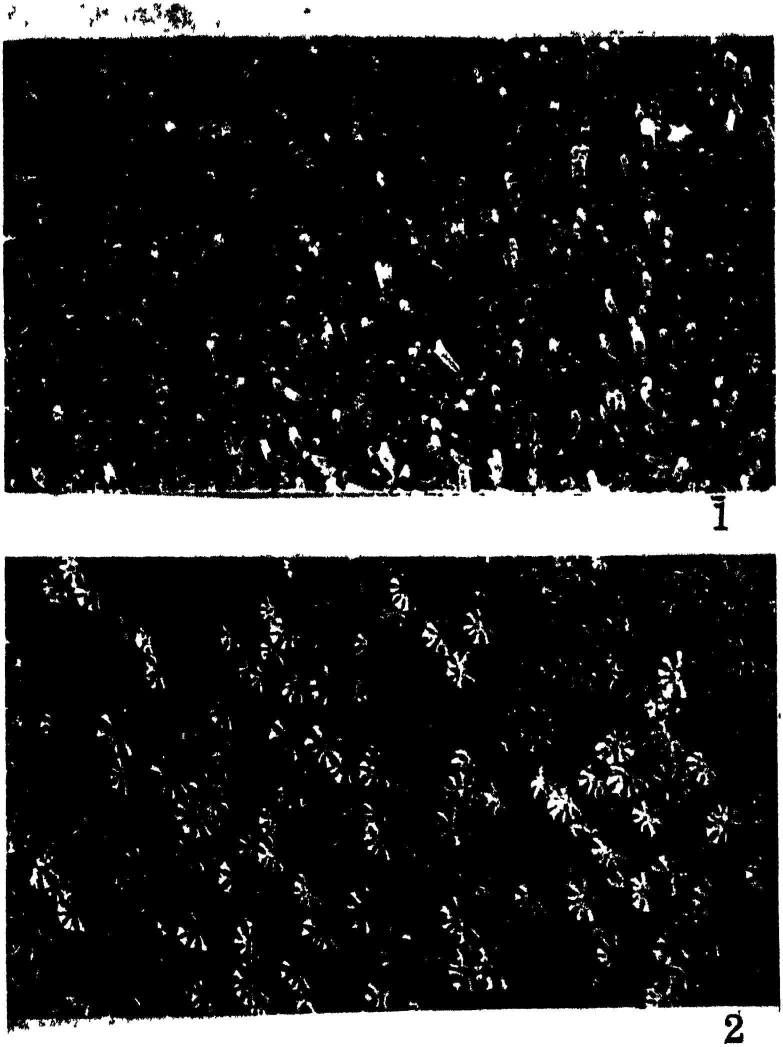
(WITH THIRTY-FOUR FIGURES)

In reviewing the literature on *Marchantia*, many studies, discussions, and references dealing with *M. polymorpha* were found As early as 1874 KIENITZ-GERLOFF (15) contributed a detailed study of it. About five years later, LEITGEB (18) made a study of *M. chenopoda* and *M. polymorpha*. CAVERS (6) contributed to another phase of *M. polymorpha*. KÜTZING (16) made a study of the elaters of *Marchantia* very early indeed. Finally, DURAND (8) speaks of *M. polymorpha* as being the most accessible as well as the most easily studied species of the genus, and feeling that it was commonly used in the laboratories, he deemed it desirable to give a somewhat complete series of illustrations and descriptions. While *M. polymorpha* has received considerable attention, *M. domingensis* has received very little. Aside from the work of EVANS (9), only one other treatise was found, which dealt with the origin of the gametophore, by LUCILE CAPT (unpublished).

It is undoubtedly true, as CAVERS (6) states, that the sporogonium affords little guidance to the phylogeny of the Marchantiales, except in a general way; nevertheless (as he also admits), taken with other characters it is of value and must receive careful study. Having available an abundance of *Marchantia domingensis* material, it was suggested by Dr. W. J. G. LAND that a study of the sporophyte be made.

Material and method

Marchantia domingensis Lehm. and Lindenb. was grown in the University greenhouse at Lincoln, Nebraska. The room was kept at approximately 22° C. and the cultures were watered nearly every day. Some of the flats were kept for several weeks under glass until near time of fruiting, when the glass was removed. The cultures grown under glass became morphologically modified. The thalli were conspicuously elongated (fig. 1), and fruiting was initiated a little



Figs. 1, 2.—Fig. 1, gametophytes grown under glass; fig. 2, gametophytes showing cupules, archegonial branches, and old antheridial branches; ×1.

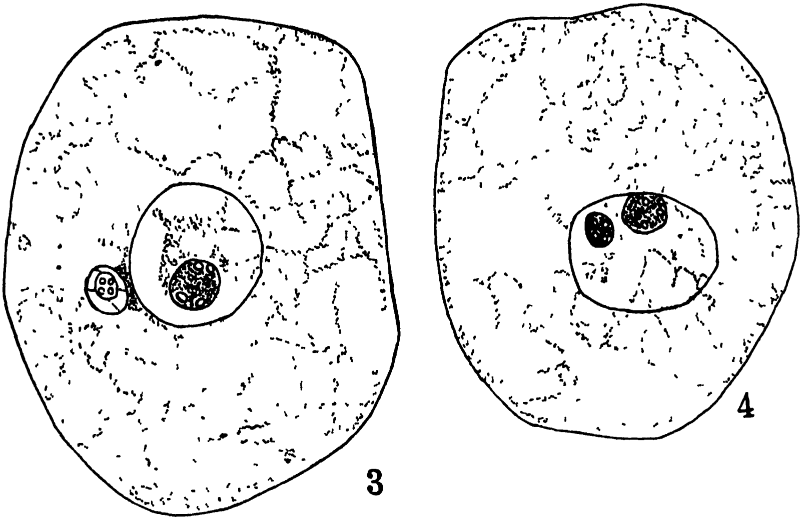
earlier than in the cultures grown in open flats; however, abundant fruit was produced in both cases (fig. 2). A few flats were given additional light by using a 100 watt mazda lamp, thus giving approximately 6 hours of artificial light in addition to the normal daylight. Cultures receiving the additional light (starting on November 22) were beginning to produce fruit on December 18, 1925. The thalli of the cultures not receiving additional light had not fruited by that time, but surpassed them in both size and color of thallus. The response of *M. domingensis* to the "long day" agrees with what WANN (24) found in *M. polymorpha* in 1922. Since the fruits from the thalli receiving only normal daylight appeared more vigorous than the plants securing additional light, they were selected almost exclusively in this study. The greater number of the figures were secured from collections made the third and fourth weeks of April, 1927. The collections were made in the forenoon and afternoon, as well as at one and three in the morning.

Material was fixed in the following solutions: acetic alcohol, Carnoy's, chromo-acetic, corrosive sublimate, Flemming's, formalin alcohol, platinum chloride, and formalin acetic alcohol. The formalin acetic alcohol and hot corrosive sublimate gave the most satisfactory results. A great number of stains were also employed, but the iron-alum haematoxylin method was for the most part used. Material was cut 5–12 μ in thickness.

FERTILIZATION

Fertilization has been reported by a number of workers in various liverworts. A remarkable similarity in the relative size of egg and sperm exists in the contact stage. GARBER (10) on *Ricciocarpus*, BLACK (2) on *Riccia*, MEYER (19) on *Corsinia*, DUPLER (7) and WOODBURN (25) on *Reboulia*, GRAHAM (11) and HAUPT (13) on *Preissia*, all show the egg nucleus about twice the diameter of that of the sperm. In *Marchantia domingensis* the egg nucleus was about three times the diameter of that of the sperm.

About thirty cases of fertilization were observed in *Marchantia*. In the first place, when the sperm has not yet come into contact with the egg nucleus, a denser mass of cytoplasm paves the space between it and the egg nucleus. This condition HAUPT (13) also illustrates in the case of *Preissia*. The egg and sperm nucleoli are very conspicuous, each being vacuolate. The egg nucleolus usually has a dense mass of chromatin-like material surrounding it, with more on one side than the other. This mass may be surrounded by another less dense material, or granular threads may protrude from it (fig. 3). Some egg nucleoli were surrounded by reticulate material (fig. 4). The sperm nucleus, especially when found outside the egg nucleus, usually had the chromatin-like material surrounding the nucleolus, but also had a few strands projecting from it (fig. 3). Centrosomes and astral rays were not seen.

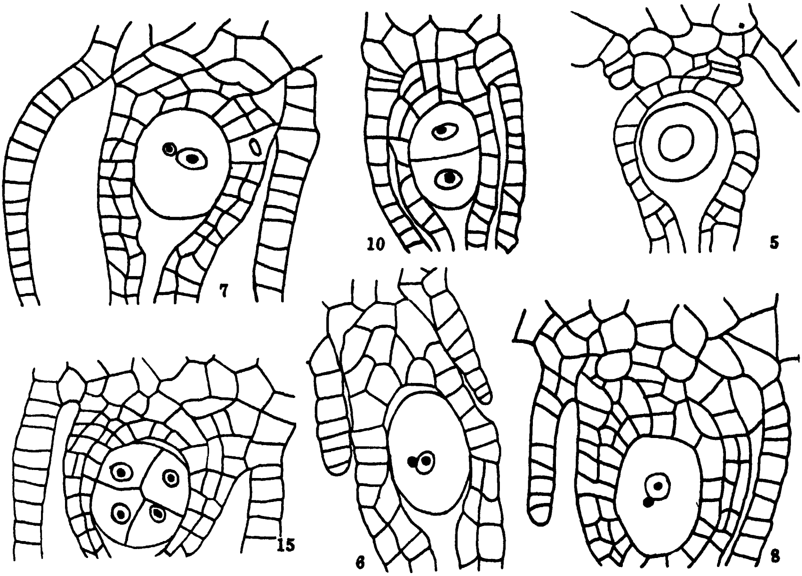


FIGS 3, 4 —Fig 3, egg with male nucleus not yet in contact with female, fig 4, male nucleus within female nucleus; ×1000.

Embryo

In the very early stage, the fertilized egg is surrounded by a venter which consists of a single layer of cells. The base of the venter and the cells beneath, composed of three or four cells in width, will be designated basal cells. The cells beneath the venter give rise to a cylindrical structure, a pseudoperianth, one layer in thickness and a few cells in height (fig. 5). A little later it is noticeable that the basal tissue, the pseudoperianth, and the venter have been stimulated to enlargement or cell division (fig. 6). The basal tissue becomes more massive, the pseudoperianth increases in length, and there occur periclinal and radial divisions in the venter, usually two successive periclinal divisions, resulting in a 3-celled structure, the calyptra (figs. 7, 8).

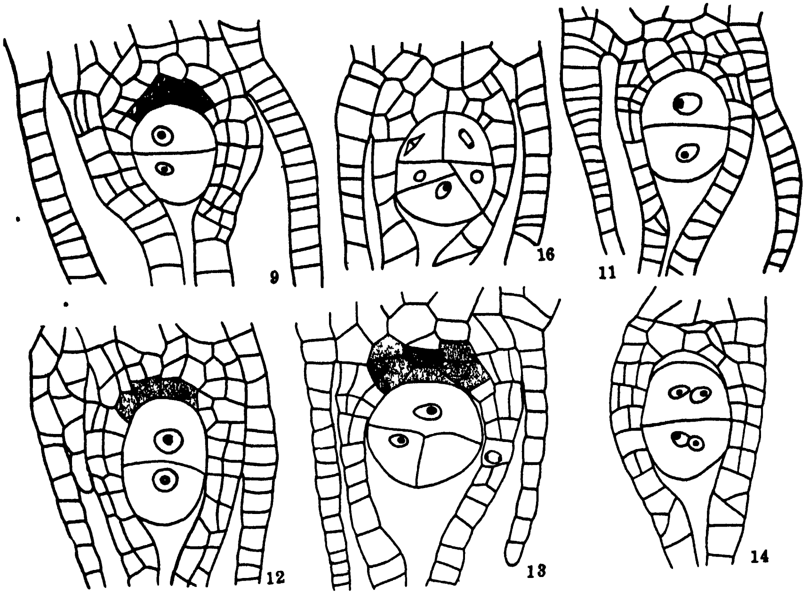
The first division of the fertilized egg gives rise to a transverse



FIGS. 5–8, 10, 15.—Fig 5, early stage of fertilized egg; fig 6, later stage of fertilization; figs 7, 8, development of surrounding structures; fig. 10, first division of fertilized egg; fig. 15, octant stage of sporophyte; ×300.

wall, which may vary in the angle that it makes with a plane which is perpendicular to the major axis of the archegonium. The time of formation of the first wall varies. In some cases it is not formed until extensive activation has taken place in all three regions, namely, the basal tissue, pseudoperianth, and venter (fig. 9); in others it may be found when the basal cells and to a greater extent the pseudoperianth have undergone several divisions, while the venter alone has remained more or less dormant (fig. 10); in still others it may occur when cell division has taken place in all three regions, relatively less, however, than was found in the first situation mentioned (fig. 11). The first row of basal cells is frequently filled at this stage with granular material (fig. 12). Very soon another row of the basal tissue may also become very granular (fig. 9).

In *Corsinia*, MEYER (19) figures the venter three to four cells thick at the time of fertilization; later, when the sporogenous tissue has been differentiated, he finds it to be in places six cells thick, with a massive neck as well. LANG (17) found in *Cyathodium foetidissimum* that with the first division of the embryo the venter of the archegonium becomes two cells thick; and when the sporogenous tissue is clearly defined by the dense and deeply staining contents of its cells, it becomes three or four cells thick.



FIGS. 9. 11–14, 16.—Figs. 9–12, variations in surrounding structures when first wall is formed; fig. 13, division of epibasal cell; fig 14, division of both cells; fig. 16, anticlinal walls in epibasal region; ×300.

CAMPBELL (4), working with *Targionia*, states that with the first divisions in the embryo, a series of periclinal walls occurs in the venter, making it two cells thick; later it undergoes further divisions, forming a calyptra four or more cells in thickness. MISS STARR (22) finds in *Aytonia* that all the cells of the venter have divided when the first division of the embryo occurs, and that growth continues in the basal tissue, the venter, and the lower part of the neck until a large amount of tissue is developed about the embryo. In DUPLER'S (7) discussion of the calyptra and involucre in *Reboulia*, he states that the former grows apace as the embryo develops, ultimately forming a several-layered and relatively somewhat massive structure. CAVERS (5) finds in *Fegatella* that fertilization is immediately followed by the appearance of tangential walls in the cells of the venter, the calyptra finally becoming five or six cells thick. BOLLETER (3), studying *Fegatella*, shows that even before the first transverse wall has appeared the calyptra has attained a wall three cells in thickness. DURAND (8); in his work on *Marchantia polymorpha*, found that while development was proceeding in the embryo, periclinal divisions began also in the walls of the venter and continued until two or three layers of cells were formed at the sides of and above the embryo.

In ascertaining the prevailing condition of the surrounding tissues in early embryogeny, it may enable us to determine the cause for the diversion of tissue in the various regions. *Marchantia domingensis* is found in some cases to differ from the related forms that have been reported, relative to the calyptra. It may pass through fertilization, form the first transverse wall, and may even attain the octant stage while the venter is still one-layered. That this factor alone may alter its subsequent development seems plausible.

After the first division of the fertilized egg, the epibasal cell divides in a plane perpendicular to the first wall laid down (fig. 13). Exceptions occur; the hypobasal may divide first, or the two may divide simultaneously, forming the quadrant (fig. 14). Following the formation of the quadrant, there occurs a vertical division of each cell, which cuts at right angles to the other two faces, resulting in the octant stage (fig. 15). This stage does not occur when the venter, basal cells, or pseudoperianth have made a definite amount of progress, but, as in the case of the formation of the first wall, its appearance is variable.

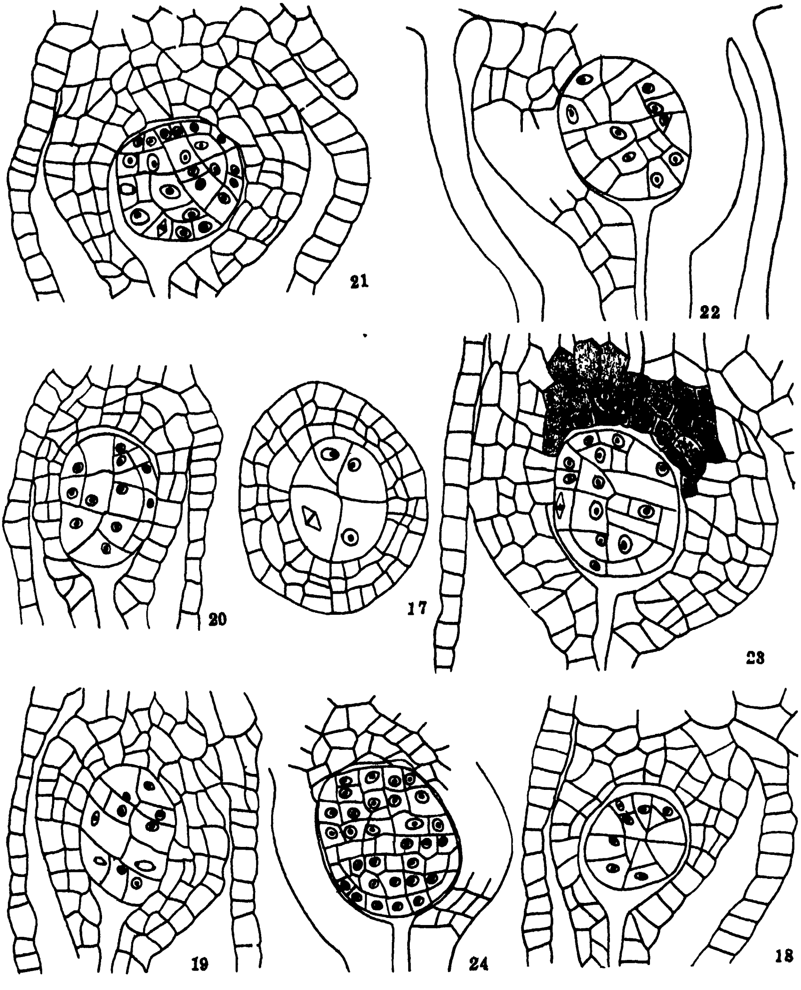
HAUPT (13), reviewing the literature on the early embryogeny of liverwort sporophytes, distinguishes two types of embryos, the filamentous and what may be called the octant type. Under the octant type appears the work on *Riccia* by Kienitz-Gerloff (14); on *Riccia*, *Targionia*, and *Hypernantron* by Campbell (4); on *Corsinia* by Meyer (19); on *Cyathodium foetidissimum* by Lang (17); on *Cryptomitrium* by Abrams (1); and on *Marchantia polymorpha* by Durand (8). To this list may be added the work of Haupt (13) on *Preissia*.

That species in the same genus do not necessarily show the same type of early development is demonstrated by the two species of *Cyathodium*. *C. cavernarum* by Lang (17) illustrates the filamentous type, while *C. foetidissimum* illustrates the octant type. *Targionia* by O'Keefe (21), *Fegatella* by Bolleter (3), and *Dumortiera* by Campbell (4) have been found to belong to the filamentous type.

Anticlinal walls make their appearance very soon after the octant stage has been formed (figs. 16, 17). The anticlinal divisions are followed by periclinal and radial walls (figs. 18, 19). Repeated cell divisions result in the formation of a somewhat globular mass. After the first anticlinal walls are formed in the octant stage, no definite procedure could be ascertained in the successive cell divisions. In studying median longitudinal sections, while the outstanding character is the large amount of irregularity displayed, there is a decided tendency toward the formation of horizontal rows in the epibasal and vertical rows in the hypobasal portion (fig. 20). The significance of this manner of cell division is obvious, especially the procedure in the hypobasal region, when subsequently it becomes very clear that it is the conducting region and much nourishment is to be transported through it. Even when the first division of the embryo forms the first wall, the basal cells full of granular material which takes a deep stain would indicate a storage region which is to be drawn upon by the developing embryo. Fig. 21 shows the greater development to have taken place in the epibasal region; on the other hand, fig. 20 shows the hypobasal region to be more developed.

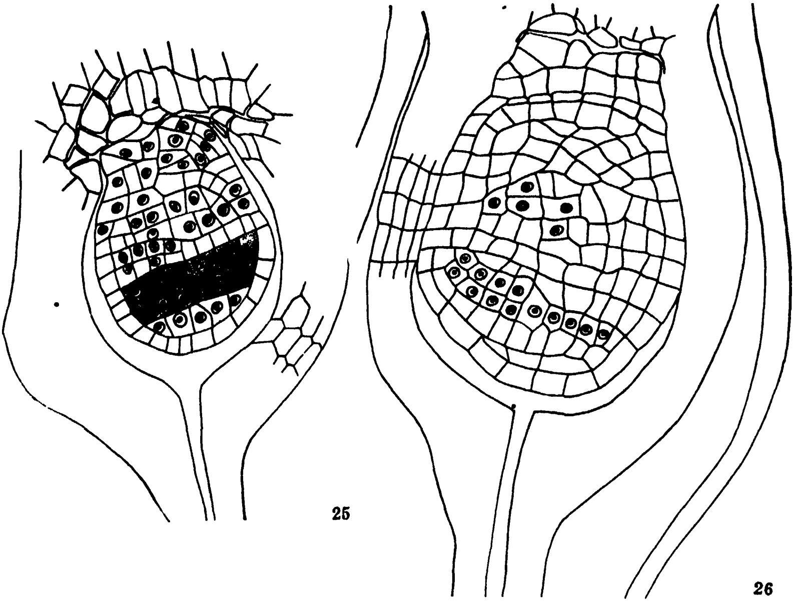
Divisions in the two upper or two lower octants do not proceed at the same rate, nor are they duplicates of each other (figs. 20–22). The calyptra divides again and becomes four cells thick.

When an outer layer of cells has been definitely cut off by periclinal divisions, it becomes the sporangium wall. In tracing the development of the epibasal region, early transverse rows of cells are found within the sporangium wall (fig. 22). At a later stage three rows of cells are found within the sporangium wall, the third row having arisen by vertical as well as by transverse divisions occurring in the second row (figs. 23, 24). Radial divisions have taken place in



FIGS. 17–24.—Fig. 17, anticlinal division in hypobasal region; figs. 18, 19, periclinal divisions in both hypobasal and epibasal regions; fig. 20, hypobasal region elongated; figs. 21, 22, stages in development of both upper and lower regions; figs. 23, 24, three rows of cells in epibasal region within sporangium wall; ×300.

the sporangium wall also. The second row of cells appears to divide and gives rise to the sporogenous tissue (figs. 25, 26). The first row beneath the sporangium wall (fig. 25) divides, producing a sterile cap or disk two cells in thickness (fig. 28). That no further division takes place in this sterile mass or cap is proved by examination of the following stages. This sterile apical cap is of common occurrence in all the upper genera of the Marchantiales, and also in the intermediate genus *Cyathodium*.



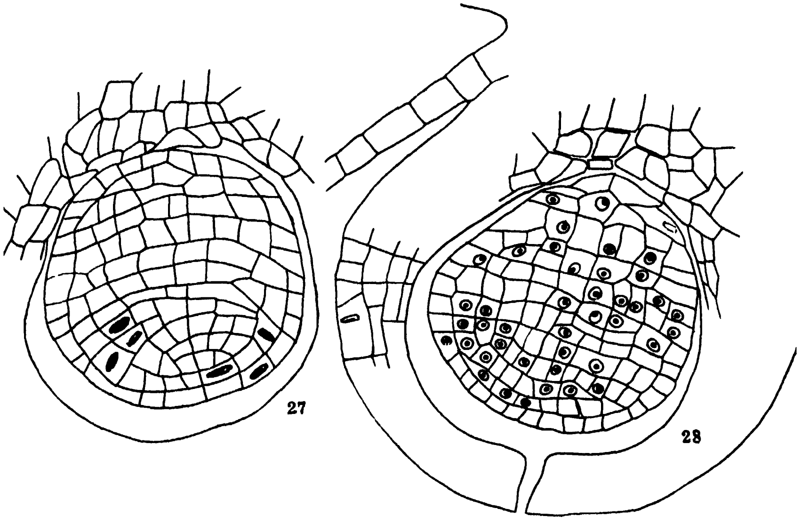
FIGS. 25, 26.—Two rows of sporogenous tissue formed from second row of cells in epibasal region; ×300.

At the time that the sporogenous tissue is being differentiated in the epibasal region, many more cell divisions have apparently occurred in the hypobasal region (figs. 24, 25). The foot has been definitely formed; the two rows of cells adjacent to the basal cells are enlarged and deeply stained, and have the appearance of basal tissue (fig. 26). The shape of the sporophyte has been modified (fig. 25). The distal end where vertical divisions are more numerous than transverse (fig. 27) tends to broaden that region, resulting in the sporophyte presenting a *Lycoperdon*-like structure.

LANG (17), in speaking of the two tiers of cells in *Cyathodium* forming an apical disk, states:

> the lowest tier can be traced back to the upper portion of the sporogenous tissues. A single layer of the latter at the upper limit of the group does not separate into spore mother cells, but remains as a continuous layer in contact with the uppermost sterile segment and forms a part of the apical disk.

In *Reboulia*, according to CAVERS (6), the apical portion of the capsule wall with its well developed cap falls away in fragments at the time of dehiscence. ABRAMS found in *Cryptomitrium* that the capsular wall at the apex consisted of two rows of cells. CAMPBELL found in *Hypernantron* that sometimes a cap of sterile cells formed the apex of the sporogonium. CAVERS (5) states that the cap in *Fegatella* appears to be derived from the uppermost portion of the sporogenous tissue. When dehiscence occurs, a line of cleavage is formed around the upper portion of the capsule just outside the apical cap. This crack is irregular and wavy, but it corresponds in general with the junction between the apical cap and the rest of the capsule wall. Valves are formed in the capsule by longitudinal splitting, but the apical cap remains, either becoming loosened all around



FIGS. 27, 28.—Fig. 27, vertical divisions occurring in epibasal region; fig. 28, sterile cap two cells in thickness; ×300.

and severed from the capsule or remaining attached to one of the valves. CAVERS (6) also finds that the apical disk not only in *Fegatella* but in *Lunularia* and *Dumortiera* is also thrown off as a lid; on the other hand, he states that in other genera the apical cap (composed of an imperfect or loose layer of cells) lies within the normally one-layered capsule wall, but the capsule dehisces by means of teeth extending to the apex. CAVERS also discusses the modified apical cap as illustrated in *Preissia commutata*, where it is a lens-shaped structure. The cells bear ringlike fibers and elater-like cells project from them.

In *Marchantia domingensis*, the two rows of sterile cells designated apical cap or disk, lying between the sporogenous layers and the sporangium wall, increase in size and resemble the granular basal tissue. They probably function as conducting tissue, since they are only one cell layer removed from the massive tissue of the archegonial neck on one side while they are in direct contact with the sporogenous layer on the other (fig. 29).

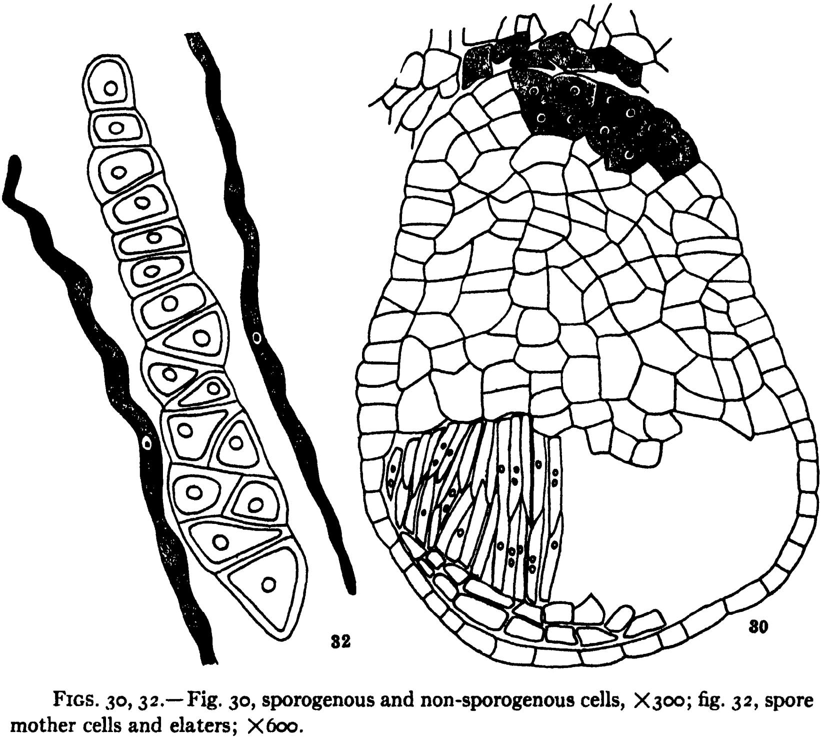
The third row of sterile cells from the sporangium wall, lying next to the first transverse wall of the fertilized egg, divides at least once in transverse section (figs. 25, 27). That it may be considered as additional tissue to the seta seems apparent. It has long been recognized by various writers that the first transverse wall divides the fertilized egg into an upper (epibasal) and a lower (hypobasal) cell. The amount of tissue in the epibasal region that has been diverted in the various genera has also received attention.

MEYER and LEITGEB both worked on *Corsinia*. They are of the opinion that the hypobasal cell contributes to the capsule in many cases at least. In *Reboulia*, HAUPT (12) found that the first transverse wall formed separates the cell which forms the foot from that which is to form the seta and the capsule. ABRAMS states that in *Cryptomitrium* usually the first transverse division is the line of demarcation between the capsule and the foot.

According to KIENITZ-GERLOFF (14), the foot and seta in *Preissia* are derived from the hypobasal cells, while the epibasal cell forms the capsule. CAVERS (5) finds in *Fegatella* that the lower (hypobasal) portion contributes only to the foot, while the stalk and capsule are both derived from the epibasal portion. In *Marchantia poly-*

morpha, DURAND (8) speaks of the first division wall as separating the stalk and capsular valves, and he believes this is usually the case; however, he states this is not always true.

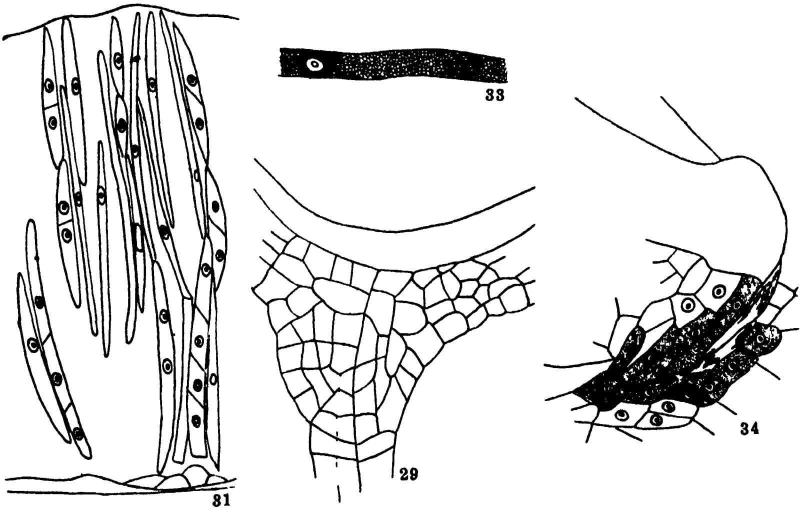
It is noteworthy that while several genera in the upper stretches of the Marchantiales have been reported to have diverted epibasal tissue, none, so far as reported, have reduced the sporogenous layers to the extent that *M. domingensis* has.



FIGS. 30, 32.—Fig. 30, sporogenous and non-sporogenous cells, ×300; fig. 32, spore mother cells and elaters; ×600.

When the sporogenous mass can be distinguished by its deeply staining cells, the two layers elongate greatly, pushing past each other to a considerable extent (fig. 30). It appeared that every other cell remained uninucleate while the alternate ones divided (fig. 30). In a stage slightly older another division had occurred, resulting in a row of four cells in each half of the sporogenous mass (fig. 31). After one more division, these cells may form very thick walls and become spore mother cells. They may divide again (fig. 32), however, making the fourth successive division since the time when practically half of it was diverted to form elaters. This situation differs from the condition in *M. polymorpha.* O'HANLON (20) states that the elaters of *Marchantia* are sister cells of sporogenous cells which undergo five divisions before the spore mother cells appear.

After the disappearance of the thick walls surrounding the spore mother cells, the usual tetrad formation is initiated. During the maturation of the spores the sporangium wall becomes strengthened by U-shaped thickenings. The elaters which at first are mucilaginous soon become granular, and remain in this condition up to the spore mother cell stage (fig. 30).



FIGS. 29, 31, 33, 34.—Fig. 29, massive archegonial neck; fig. 31, second division of sporogenous cells and uninucleate elaters; fig. 33, elater containing peripheral layer of minute cytoplasmic vacuoles; ×600; fig. 34, half of longitudinal section of foot showing elongated lateral cells; figs. 31, 29, 34; ×300.

At the time of the disappearance of the spore mother cell wall, the elaters show a peripheral layer of minute cytoplasmic vacuoles (fig. 33). As elongation in the elater proceeds, larger vacuoles occur between bands of smaller ones. Later, in place of the small cytoplasmic vacuoles, spiral thickenings occur. This situation confirms the finding of STOVER (23), who states that pitted thickenings arise by the vacuolation of the cytoplasm, the thickening being laid down by the thicker portion of the cytoplasm. He also believes that the type of tracheae varies with the amount of elongation.

FOOT AND SETA

Since the basal tissue becomes granular when the first transverse wall of the embryo is formed, it indicates that this is the region which contributes the greatest amount of nutrition to the developing embryo (fig. 9). It is the region through which the nutrition is constantly moving. On the sides where the seta makes contact, and above where the sporangium wall may come in contact with the calyptra, it is intermittent, due to differential growth. Later the space between the seta and the calyptra increases. The lateral cells of the foot (fig. 34) which keep in contact with the gametophyte become greatly elongated, changing the shape of the basal portion. The tendency for the seta to elongate vertically and the cells of the foot to become greatly elongated gives the pileus-shaped foot. The lateral cells of the foot, which at first elongate, soon undergo transverse division. The cells of the seta, arranged in rows, connecting the much enlarged cells of the foot with the sporogenous mass, contain vertical walls which appear thicker than the transverse walls. The cells do not lose their nuclei. The two deeply staining rows of the foot of *M. domingensis* show a marked contrast to the illustration as given by DURAND (8) in *M. polymorpha,* where all the cells of the foot appear to be deeply staining. At an early stage, particularly when the sporogenous tissue has undergone two divisions in *M. domingensis*, the seta is found to be much greater in length than it is in *M. polymorpha* at this stage. The cells of the former, constituting the seta, display greater linear arrangement than similar cells in the latter do. Whether these minute differences in the tissues are indicative of greater specialization may at least be questioned.

Summary

1. At the time of fertilization of *Marchantia domingensis* both egg and sperm nucleoli are very conspicuous. Chromatin-like material surrounds the nucleolus of the egg, while granular strands usually project from the sperm.
2. The time of the appearance of the first wall varies.

3. The early development of the sporophyte is the octant type which occurs in the greater number of the Marchantiae.

4. The epibasal cell gives rise to three regions, namely, an upper sterile region consisting of two rows of cells forming the apical cap; beneath the cap the two sporogenous layers; and underneath them another sterile region contributing to the seta.

5. The hypobasal cell forms the remainder of the seta and foot.

6. The apical cap functions as a conductive system between the massive neck and the sporogenous tissue.

7. The foot early differentiates two rows of deeply staining cells that retain this condition even after the tetrad formation.

8. The vertical walls of the seta appear thicker than most of the transverse walls, although they do not lose their nuclei.

9. The venter may not begin tangential divisions until after the octant stage has been formed.

10. Sporogenous cells undergo three or four divisions before spore mother cells are formed.

11. Sporogenous tissue consists of two transverse rows, practically half of which become sterile forming elaters.

12. Vacuolation of the peripheral cytoplasm in the granular elaters is responsible for the spiral thickenings occurring later.

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MORPHOLOGY OF NORTH AMERICAN SPECIES OF POLYGALA

THEO. HOLM

(WITH FORTY-TWO FIGURES)

Introduction

The absence of internal secretory organs is, according to SOLEREDER (7), characteristic of the Polygalaceae, with exception of the lysigenous secretory ducts and oil cells of many South American species of *Polygala*, which have been mentioned by CHODAT (1, 2), and which SOLEREDER recommends to be reexamined as well as the occurrence of peculiar granular masses resembling aggregated crystals, which VESQUE (8) found in the leaves of certain Brazilian species of *Polygala*. The discovery of these peculiar crystal-like bodies in the Brazilian species was compared by VESQUE with the epidermal secretions in violets from Chile, and he reached the conclusion:

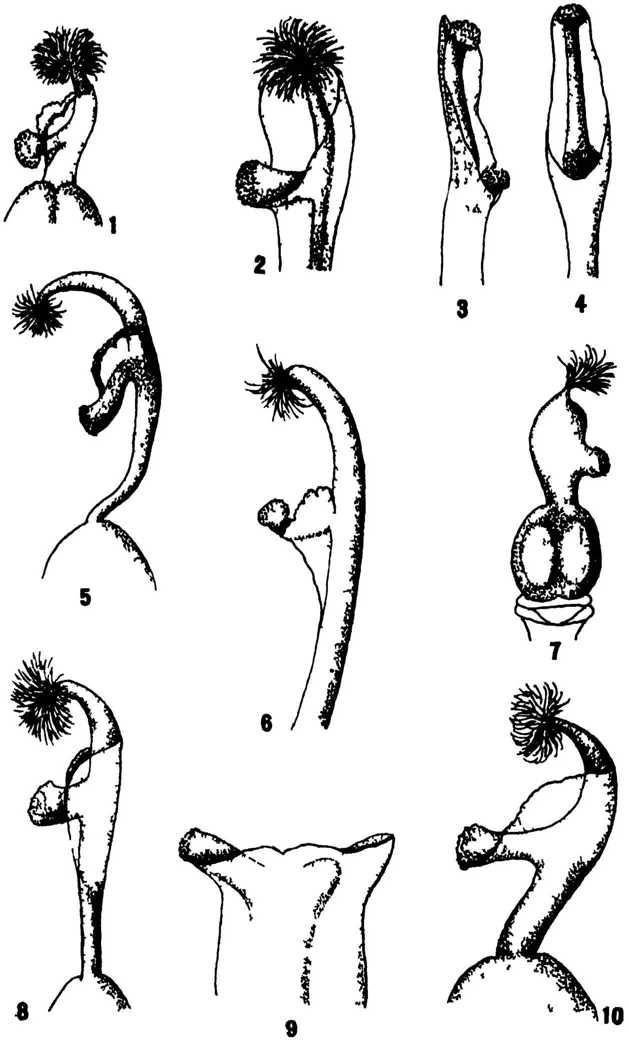
> Si nous considérons ces faits au point de vue de l' anatomie comparée, nous voyons que ce genre (*Polygala*) se comporte comme les *Viola* dont certaines espèces géographiquement limitées se distinguent également pai une sécrétion particulière.

With regard to the Chilean violets, the discovery made by VESQUE was corroborated by REICHE (5), who had considerable material at his disposal, but so far as concerns the lysigenous ducts mentioned by CHODAT in *Polygala*, these have not been observed in other species. An anatomical study of *Viola* and *Polygala*, as these genera are represented in Maryland and Virginia, has shown that several species of the former show a structure corresponding with that of the Chilean species, so far as concerns the secretions, and that several species of *Polygala* do possess lysigenous ducts in leaves as well as in stems.

While studying *Polygala*, however, it was noticed also that the floral structure is quite characteristic in certain species, although not described in any very precise manner in the various botanical manuals. For instance, the structure of the style and stigmata is difficult to describe without figures, therefore some of the types represented in my material are illustrated here. I have examined the following species of the section Orthopolygala Chodat: *P. polygama* Walt., *P. senega* L., *P. incarnata* L., *P. ambigua* Nutt., *P. curtissii* Gray, *P. mariana* Mill., *P. nuttallii* Torr. & Gr., *P. sanguinea* L., and *P. lutea* L.

STYLE AND STIGMATA

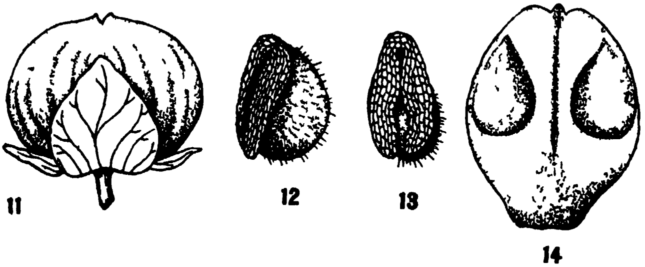
The apex of the style is bicleft, and generally very unequally so. The posterior branch, the shorter one, has a typical, papillose, viscid stigma; while the anterior is terminated by a tuft of long, bifurcate hairs, but with no stigma. A very thin membrane is very conspicuous; in *Polygala lutea* (fig. 5) this covers only the upper face of the stigmatic lobe, while in *P. polygama* (fig. 10) it extends around the basal part of both style branches. In *P. incarnata* the membrane is confined to the posterior face of the style, extending from the stigmatic lobe a short distance beneath this. In *P. curtissii* (fig. 8) the membrane covers the base of both style branches, extending a short distance downward, and this structure recurs in *P. mariana* and *P. nuttallii*. In *P. ambigua* the relatively short style is surrounded by the membrane, which extends upward to near the apex of both style branches, a structure which recurs in *P. alba* Nutt. and *P. paniculata* L. In *P. senega* (fig. 7), on the other hand, there is no membrane, but otherwise the structure resembles that of the other American species. In *P. longicaulis* H.B.K. (fig. 2), collected in Porto Rico, the membrane is very long, extending above the apex with the hairy appendage, and a similar development of the membrane recurs in *P. tenuifolia* Willd. (figs. 3, 4) from Nerczynsk, Dahuria, where the anterior style branch is terminated by a globose, papillose, but not viscid stigma, while no tuft of hairs is developed. Finally, in *P. paucifolia* Willd. of the section Chamaebuxus DC. (fig. 9) the membrane forms a tube almost half as long as the whole style, and only the viscid stigma is visible, the barren branch of the style being very short and hidden within the membrane. So far as concerns the North American species, *P. paucifolia* and *P. senega* may be readily distinguished from the others; but with reference to those with membrane and a tuft of hairs, the difference depends only on the varied development of the membrane: small in *P. lutea* and *P. incarnata*, larger in the others. The difference in the



Figs 1 10 —Fig 1 *Polygala ambigua*, upper part of ovary with style and stigmata, fig 2, *P longicaulis*, upper part of style with stigmata, fig 3, *P tenuifolia*, upper part of style with stigmata side view, fig 4, same, upper part of style with stigmata, front view, fig 5, *P lutea*, upper part of ovary with style and stigmata, fig 6, *P incarnata*, upper part of style with stigmata, fig 7 *P senega*, ovary with style and stigmata, fig 8, *P curtissii*, upper part of ovary with style and stigmata, fig 9, *P paucifolia* upper part of style with stigmata, fig 10, *P polygama*, upper part of ovary with style and stigmata, ×30 style and stigma structure is much more pronounced in the South American species, when we compare the numerous structures figured by Chodat. In this respect the southern members of *Polygala* agree with the Andine violets, of which Reiche has figured a number of very singular structures exhibited by the style and stigma.

Fruit

The fruit is a compressed, 2-celled capsule, wingless in the species enumerated; seeds are solitary in the cells, pendulous, more or less hairy, and conspicuously carunculate. Among the types of fruit and seed characteristic of some of the species may be mentioned: the thickish capsule, much broader than long in *P. senega* (fig. 11), and the caruncle as long as the seed itself (figs. 12, 13); the large, thin-walled capsule of *P. sanguinea* (fig. 14) notched at the apex; the oblong capsule of *P. incarnata* (figs. 17, 18), and the caruncle about half as long as the seed itself (fig. 19); the small capsule of *P. ambigua* (figs. 23, 24), and the seed with its relatively short caruncle (fig. 25); the large, turgid capsule of *P. mariana* (fig. 29), and the distinctly apiculate seed with a short caruncle (figs. 30, 31); and the oblong capsule of *P. lutea* (fig. 34), notched at the apex, and the seeds with a long caruncle (figs. 36, 37). Characteristic of *P. lutea* also is the carpophore (fig. 35), distinctly 2-celled, which remains together with the wings, after the capsule has opened and the seeds dropped out; in all the other species examined the flowers fall off completely when the fruit has matured. The fruit and the seed thus show some specific differences, notably in *P. senega*, *P. sanguinea*, *P. mariana*, and



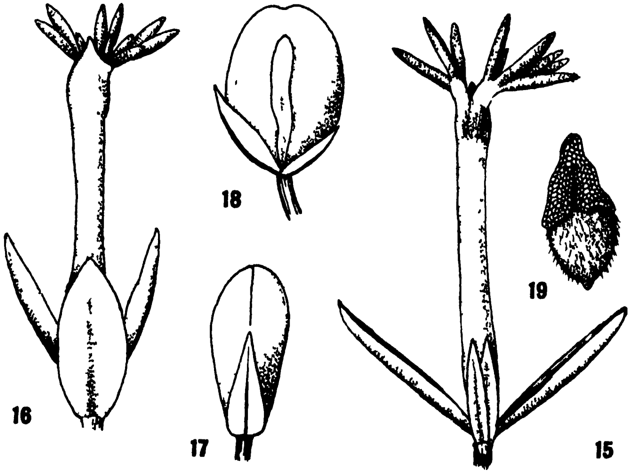
Figs 11–14.—Fig 11, *Polygala senega*, capsule with sepals, side view, ×14, figs. 12, 13, same, seeds, side and front view, ×20, fig 14, *P. sanguinea*, capsule, side view, ×14.

P. lutea; while the structure shown by *P. incarnata* and *P. ambigua* recurs in several of the other species of the section Orthopolygala.

CALYX AND COROLLA

The flowers are strongly zygomorphic (the plane of symmetry being median); they have five free sepals, the two lateral ones of which are large, petaloid, and frequently project on each side like the "wings" of a pea flower; the other sepals are much smaller, sepaloid, and the odd one (dorsal) is generally a little longer than the anterior pair. There are five petals, of which the two lateral ones are either rudimentary or completely absent, as in our species, and the anterior (keel) is large, hollow, and carinate with the apex lobed or fimbriated, and more or less connate with the others. The shape, size, and color of the wings and petals show several distinct characters in the genus, even in the small number of species I have examined.

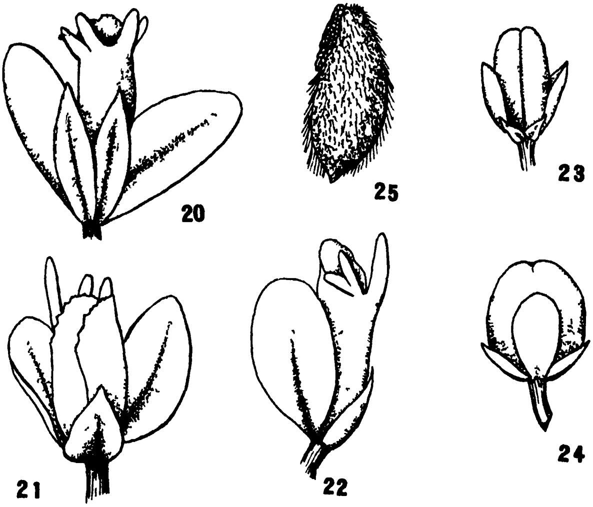
In *Polygala incarnata* (figs. 15, 16) the wings are relatively short, spatulate, and green with broad hyaline margins. The petals are united into a long slender tube, pale rose or purplish, and twice as long as the wings; the keel is crested at the apex in the manner of four bicleft, narrow lobes with two additional pairs, but much shorter, all spreading, and of a deep purplish color. In *P. senega* the small flowers are greenish white; the three small sepals are relatively broad and obtuse, and the wings are also broad, obtuse, and a little longer than the corolla. The petals are almost free, the two posterior being oblong, obtuse, and about as long as the broad keel with its crest of about ten short, obtuse lobes. In *P. ambigua* (figs. 20-22) the very broad, obtuse wings are a little shorter than the connate petals, of a white color with green midveins. The petals are pale greenish, and the small crest consists of one pair of short lobes. In *P. mariana* (figs. 26-28) the wings are relatively narrow, acuminate and unguiculate, purplish, one-nerved, and of about the same length as the corolla; the two posterior petals form a tube around the keel and are distinctly longer than this, including the pair of short, bifid lobes. While the tube of the two petals is pale yellow, the keel is hyaline with the crest deep yellow, but dark purplish when fading. The flower of *P. nuttallii* resembles that of the preceding species, but the wings are of a paler purplish color. In *P. sanguinea* the wings are ovate-oblong, purplish above, whitish at the base, and the keel is white. In *P. lutea* the wings are 5-nerved, ovate-oblong, with a short, sharp, apical cusp, and longer than the petals; of these the two lateral form a tube, more or less emarginate at the apex (figs. 32, 33). The keel bears a crest of four bifid, short narrow lobes. The wings and the petals are of a beautiful orange color, and, as already stated, the wings persist for a long time after the fruit has matured and the petals have dropped off. The color of the flowers does not turn dark in drying. Characteristic of these species is thus the constant development of a crest upon the keel; the long tubular corolla of *P. incarnata* makes this species very distinct from the others. In the section Chamaebuxus the keel varies from simply rostrate to crested, and in *P. paucifolia* the crest is very conspicuous and plumose; but with regard to the stigmatic structure *P. paucifolia* differs from the species of Orthopolygala as previously described.



Figs. 15–19.—Fig. 15, *P. incarnata*, flower, front view, fig 16, same, flower, back view; fig. 17, same, capsule, back view, fig. 18, same, capsule, side view, fig 19, same, seed, front view, ×10.

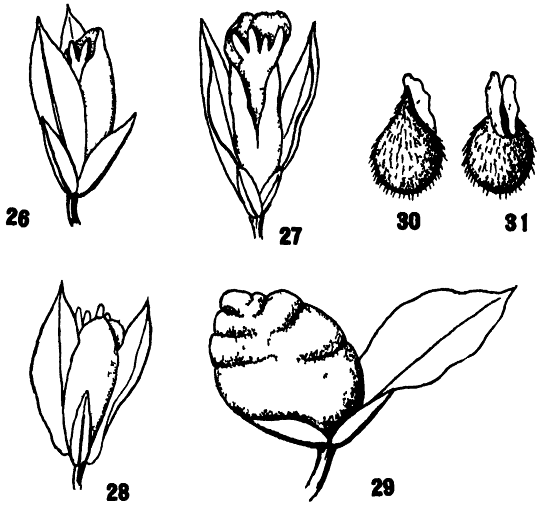
Vegetative reproduction

Of the species examined, *Polygala senega*, *P. polygama*, and *P. lutea* are perennial, the others annuals. There is a very gradual transition from the annual habit to the perennial, and this transition is well exemplified by *P. lutea*. The occurrence of *P. lutea* in the vicinity of Clinton, Maryland, was rather unexpected, because it was known only from a single station near Washington, D.C., a swamp near Suitland, about 10 miles from Clinton; the writer had for several years studied the vegetation in the many swamps near Clinton, but without seeing a single specimen of this characteristic and very conspicuous species. A gravel bank bordering on a sphagnum swamp was dug out during the fall some years ago, and the surface, left open, was completely without any vegetation. In the succeeding spring, however, several seedlings appeared, mostly Gramineae, but beside these some small seedlings appeared with epigeic cotyledons, and rosettes of broad, roundish leaves of a light green color. Early in May these seedlings reached the flowering stage, and proved to be *P. lutea*. Among the Gramineae with which it was associated was the very rare *Panicum clutei* Nash, not hitherto observed in this vicinity. How these rare plants had reached this locality seems difficult to explain, unless the seeds had been buried in the ground for several years without losing their vitality. That



Figs. 20–25.— Figs. 20–22, *P. ambigua*, flowers seen from front, back, and side, ×14, figs. 23, 24, same, fruits, seen from front and side, ×12, fig. 25, same, seed, side view, ×30.

grasses may act in this manner is a fact well known, but in regard to *Polygala* it seems very surprising, since the seeds of this genus lose their vitality very soon when collected and kept dry. Nevertheless, I have had the same experience with *P. ambigua*, almost as rare as *P. lutea* in this vicinity, which suddenly appeared in great abundance in a field ploughed over. But returning to *P. lutea*, the seedlings thus reach the flowering stage during the spring, and the leafy rosettes continue to develop leaves and to increase in size. The fruits matured in July, and from the seeds a second vegetation soon appeared, developing flowers and fruits during the fall, as late as the beginning of November. The rosettes of the plants from May, as well as the later ones, kept fresh during the winter, producing flowers again the succeeding spring. The duration of these plants was observed to extend over three years; thus the species cannot be said to be either annual or biennial, as stated in the manuals. Moreover my herbarium includes some specimens from New Jersey, which show withered stems from the year preceding; thus it seems natural to suppose that the species retains its perennial habit also at stations farther north. The species has no rhizome, but the primary axis per-



FIGS. 26–31.—Figs 26–28, *P. mariana*, flower, seen from side, front, and back, ×14, fig. 29, same, fruit with one wing, ×14, figs 30, 31, same, seeds, side and front view, ×20.

sists, increases in thickness, and bears a leafy rosette with axillary buds. The root system consists of several relatively strong, secondary roots, beside the primary, which remains slender, of a light brown color. We have thus in *P. lutea* a plant of exactly the same habit as *Viola tricolor*, described by WITTROCK (10), one of the several analogies existing between these two genera.

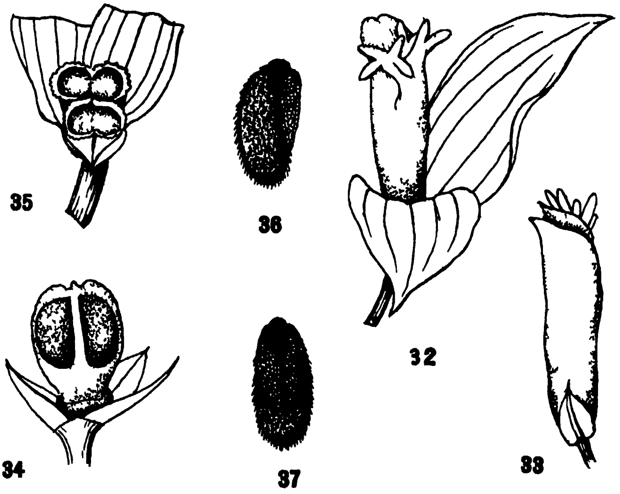
A much stronger perennial habit is represented by *P. polygama* and *P. senega*, in which the primary root persists as a "radix multiceps," increasing in length and thickness, and being crowned by a number of aerial floral stems, which die off at the close of the season, but leave the basal internodes active with their axillary buds. It is a typical pseudo-rhizome: complex of persisting stem bases, but with no rosette of leaves. In *P. polygama* subterranean stems develop from the axils of the lowest leaves, bearing cleistogamic flowers, more fertile than the aerial chasmogamic ones. A similar but less strongly developed pseudo-rhizome recurs in *P. alba*, *P. cymosa*, and the Dahurian *P. tenuifolia*.

Polygala paucifolia is also perennial, but the herbarium material is not sufficient to show the primary development of the deep-seated pseudo-rhizome with its slender roots. The stems do not grow directly upward to the light, but are horizontally creeping beneath the surface for some distance before they develop into erect, flower-bearing stems. These subterranean stems have small scalelike leaves, and they root at the nodes; some of them branch and bear cleistogamic flowers. None of the stems, neither the subterranean nor the aerial, persist for more than one season. This same habit recurs in *P. cornuta* Kell., but the subterranean stems become woody, and the aerial shoots partly persist, thus representing an undershrub. The European *P. chamaebuxus* shows a corresponding habit. In *P. californica*, on the other hand, the root system consists of a large, woody, branched taproot crowned by the persisting stems, thus representing a typical "suffrutex." WARMING (9), who has contributed so much to our knowledge of the biology and structure of Scandinavian plants, described the development of the pseudo-rhizome (radix multiplex) of *P. vulgaris* as follows:

> The thin, decumbent stems proceed from an almost leafless center, and the primary shoot dies off except the basal portion; the cotyledonary buds develop

into decumbent shoots, which ramify, and of which the basal portion persists; the primary root is relatively slender, but persists, and no secondary roots become developed.

Thus in *Polygala* the annual habit passes gradually into the perennial, and from the habit "annual" the transition into "perennial" is well exemplified by *P. lutea*. But none of the North American species develop any further than becoming "undershrubs."



FIGS. 32–37.—Figs. 32, 33, *Polygala lutea*, flower, partly side and partly back view, ×14, fig. 34, same, fruit, side view, ×14; fig. 35, same, base of fruit, carpophore, front view, ×14, figs. 36, 37, same, seeds, side and front view, ×20.

INTERNAL STRUCTURE OF VEGETATIVE ORGANS

ROOT SYSTEM.—The very thin lateral roots of *Polygala lutea*, growing in sandy soil near a creek, have no hairs, but the epidermis is papillose. There is no exodermis, and the cortex consists of three compact layers of large-celled, thin-walled parenchyma. The endodermis is thin-walled, and the pericambium remains intact; the stele is triarch, and the center is occupied by a narrow strand of thick-walled conjunctive tissue. No oil ducts were observed, but many of the cells of the cortex contained a dark granular substance. The primary root shows an early increase in thickness, the pericambium having developed a few strata of secondary cortex but no cork. The stele shows a continuous band of leptome and hadrome in deep rays;

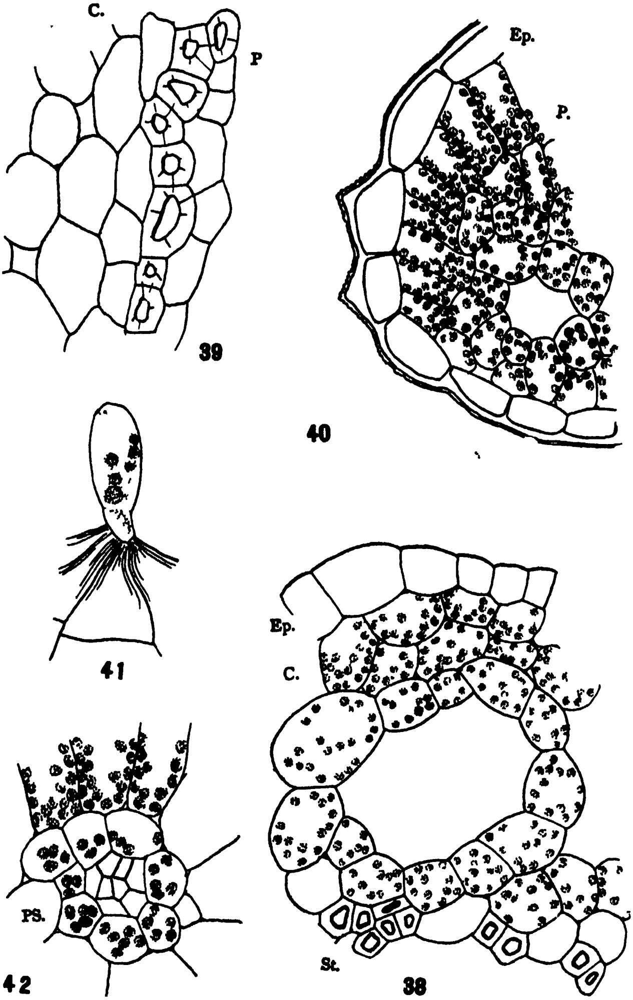
no oil ducts were observed. In the annual species, for instance *P. mariana*, the primary root and its strong lateral ramifications show four to five thin-walled strata of pericambial cork, while all the peripheral tissues from epidermis to endodermis have been thrown off. The stele shows the same structure as the primary root of *P. lutea*, but contains many strata of thick-walled libriform; no oil ducts were observed. A very singular structure is exhibited by *P. senega*, at least in the older roots, as described by the writer (3) in a previous paper. In very young roots the structure is normal, and shows the regular development of all the tissues. In these is a sparingly hairy epidermis, and a cortex of four strata, thin-walled but compact, and with a yellowish substance in some of the cells. The endodermis is thin-walled, and the pericambium surrounds four short rays of hadrome, and four exceedingly small strands of leptome. The vessels and the conjunctive tissue are yellow and thick-walled; no pith is developed. When the roots grow older the structure becomes very much changed, not only on account of the increase in thickness, but also on account of the very irregular increase, so as to make the stele excentric.

By the gradual growth in thickness, the epidermis, cortex, and partly also the endodermis become thrown off, while strata of cork arise from the pericambium. A secondary cortex develops also from the pericambium, and secondary leptome and hadrome begin to form collateral mestome strands, separated from each other by rays of parenchyma. If this increase in thickness due to these secondary tissues were uniform all round the center of the root, we should have a normal and very frequent root structure, but the increase is not regular. The secondary cortex is much thicker on the one side of the root than on the other, and the mestome strands are much broader, that is, they contain a much greater number of vessels in each row than on the other side. Furthermore, the parenchymatic rays are long and narrow on the one side of the root, but very short and enormously broad on the other. It is on that part of the root where the cortical parenchyma is so strongly developed that the characteristic keel becomes formed, when the root is dried. A cross-section of the root thus shows an excentric stele of an elliptic to ovate outline, and a cortex which is thickest outside the broadest mestome strands. Where the mestome strands are short and the parenchymatic rays very broad, the cortex occupies a very narrow zone of the cross-section. With regard to the minor structure of the various tissues, the cork consists of about four layers of a brown color. The secondary cortex may consist of about twenty layers on the one side of the root, but of only ten or less on the other; the cells are slightly thick-walled, and arranged radially toward the center of the stele. No starch was observed, but a yellow substance occurs frequently in this parenchyma, although not in specialized ducts or reservoirs of any kind. The cell walls of the cortex are also yellow in some places. Small strands of leptome occur in the cortex some distance from the main stele. In the stele is leptome, cambium, and hadrome; the latter containing many wide vessels, and the parenchymatic rays varying in length and width, as just stated; the cells of these rays are often thick-walled. In the vessels and in the adjoining parenchyma is frequently a yellowish, somewhat oily substance, but only in the root, not in the stem and leaves. While thus the most common irregularity in the increase in thickness depends on the one-sided development of the secondary tissues, several other irregularities occur, and have been described by LINDE (4). For instance, the cortex may form two broad wings, one on each side of the root. The stele may be divided into several broad rays of mestome with broad parenchymatic rays; or it may be divided into two separate steles as if two roots had grown together. But whatever irregularities be observed, it seems to be constant that no pith is developed, and that the primordial stele becomes preserved throughout the life of the root. Very characteristic of the root of *P. senega*, however, is the development of a band of leptome strands outside the stele, which is not mentioned by CHODAT. This investigator, on the other hand, observed in *P. obovata* St.-Hil. that the hadrome was divided into a great number of strands of very irregular outline, as in the tuberous roots of *Althaea*, *Scorzonera*, *Daucus*, etc. We have thus in *Polygala* two remarkable cases of root structure, exemplified by *P. senega* and *P. obovata*.

AERIAL STEM.—The stem is cylindric in *P. senega*, obtusely five-winged in *P. incarnata* and *P. lutea*, sharply four-winged in *P. nuttallii*, and sharply five-winged in *P. mariana*, *P. ambigua*, *P.*

curtissii, *P. sanguinea*, and *P. polygama*. The cuticle is relatively thick, but perfectly smooth in *P. lutea*, and also in *P. senega* except where it covers the short curved hairs in the form of pearls; in the other species the cuticle is distinctly wrinkled longitudinally, and especially in the wings. The epidermis is thin-walled in *P. lutea*, and short, unicellular, obtuse hairs are quite frequent; in the other species the outer cell wall of the epidermis is more or less thickened, and especially in the wings, where it is raised so as to form papillae. The lumen of the cells in *P. polygama* is very wide all around the stem. Oil drops were observed in many cells of the epidermis in *P. incarnata*, *P. lutea*, *P. mariana*, and *P. nuttallii*, but not in the four other species, although *P. sanguinea* has oil ducts in the cortex. With regard to the cortex, this tissue is homogeneous in *P. lutea*, *P. polygama*, and *P. senega*, consisting of several, three to six strata of isodiametric cells; while in *P. sanguinea* some palisade cells occur here and there, and a small strand of collenchyma was observed in the sharp wings of *P. polygama*. In the other species examined (Orthopolygala) the peripheral one or two strata of the cortex represent typical palisades, the inner more or less roundish cells. Lysigenous oil ducts were observed in the cortex (fig. 38), and they are surrounded by a ring of parenchyma, the lumen and shape being more or less distinct from that of the surrounding cortex. Their distribution is very regular, corresponding with the outline of the stele, which is pentagonal. In *P. lutea* there are thus ten ducts, five outside the five corners of the stele and five in the spaces between these. In *P. mariana*, *P. nuttallii*, and *P. sanguinea* the five ducts are located outside the five corners of the stele.

An endodermis was observed only in *Polygala senega*, and it was not very distinct; a pericycle, on the other hand, is represented in all the species examined, and it is composed of two or three strata, the peripheral being stereomatic, the inner thin-walled and parenchymatic (fig. 39). The stele itself shows five primary, collateral mestome strands, between which a cambium develops strands of leptome and libriform, but no vessels. This cambium corresponds with the intrafascicular, and the pericycle takes no part in developing the secondary mestome strands. The oil cells, which CHODAT claims to have found in the wood of *P. senega*, were not observed in this material. The pith is thin-walled and contains neither starch nor crystals of any kind.



Figs. 38–42.—Fig. 38, cross-section of stem of *P. lutea* showing wide duct in cortex (*c*); *ep*, epidermis; *st*, stereomatic pericycle; ×360; fig. 39, cross-section of stem of same species; *c*, cortex; *p*, pericycle of two strata; stereomatic and parenchymatic; ×360; fig. 40, cross-section of leaf of *P. mariana* showing duct in chlorenchyma, beneath palisade tissue (*p*); *ep*, epidermis; ×360; fig. 41, hair from leaf of *P. lutea;* ×480; fig. 42, cross-section of leaf of *P. incarnata* showing one of the lateral veins with parenchyma sheath (*p s*); ×480.

LEAF.—*Polygala senega* is the only one of the species examined of which the leaf structure is dorsiventral with reference to the stomata, being confined to the dorsal face, and the chlorenchyma being differentiated into a ventral palisade and a dorsal pneumatic tissue. In the other species stomata occur on both faces of the leaf blade, and the chlorenchyma is mostly represented by palisade cells in the ventral as well as in the dorsal part of the leaf.

The cuticle is thin and smooth all over the leaf blade in *P. incarnata*, but wrinkled in the other species, especially where the epidermis is papillose (fig. 40). The epidermis is very thick-walled (the outer wall) in *P. incarnata*, thin-walled in the other species except in the midrib and margins. The stomata are level with the epidermis, and hairs of the type described for the stem are frequent on both faces of the leaf in *P. lutea* and *P. senega*, especially above and beneath the stronger veins. Viewed in superficial sections the lateral walls of the epidermis are nearly straight on both faces of the blade in *P. lutea* and *P. incarnata*, but undulate on the dorsal face in the other species. As already mentioned, the palisade tissue in *P. senega* is confined to the ventral part of the leaf, and the same structure recurs in *P. lutea*, *P. curtissii*, and *P. sanguinea*, but in these the stomata are distributed over both faces of the leaf. In the other species the pneumatic tissue is inclosed by the ventral and dorsal strata of palisade cells. With regard to the occurrence of oil in the leaves, oil drops were found in the epidermis of *P. lutea*, *P. mariana*, *P. nuttallii*, and *P. sanguinea*. Lysigenous oil ducts were observed in the chlorenchyma between the veins, and between each two of these, in *P. lutea*, *P. mariana* (fig. 40), and *P. sanguinea*, while only one single duct was seen in *P. incarnata*. No oil drops nor ducts were found in *P. curtissii*, *P. ambigua*, *P. senega*, nor *P. polygama*, neither in the stems nor in the leaves.

The mechanical tissues, stereome and collenchyma, are almost absent; in *P. senega* there are a few collenchymatic cells beneath the dorsal epidermis, and in *P. ambigua* the midrib has a small support of rudimentary stereome on the leptome face. Water storage tissue is also poorly represented in the dorsal keel of *P. senega*, *P. polygama*, and *P. lutea* (stem leaves). The median mestome strand is crescent-shaped in cross-section, and more or less imbedded in the chlorenchyma. The lateral veins (fig. 42) are generally very thin, and surrounded by thin-walled, green parenchyma sheaths.

PETIOLE.—There is a distinct but relatively short petiole in *P. senega;* it is crescent-shaped in transverse sections, and hairy like the stem and leaf blades. Many of these hairs are borne on small cushions of epidermal cells, however, and are frequently curved. The cuticle is prominently wrinkled, and the epidermis is thick-walled. A collenchymatic tissue occurs on the ventral face beneath the epidermis. The single mestome strand is surrounded by a large colorless parenchyma, but no stereome is represented.

These species of *Polygala* thus exhibit several peculiar structures in the roots, as well as in the stems and leaves. In the root of *P. senega* the presence of a band of leptome strands outside the stele may be seen. A yellowish, oily substance was observed in the root of this species, in the secondary cortex, also in some of the vessels and the adjoining parenchyma, while no oil was found in the leaves or stems. In several of the other species oil drops were found in the epidermis of stem and leaves, and lysigenous oil ducts were also observed in several species, in the leaves between the veins, in the stem outside the corners of the pentagonal stele (in cross-sections), and sometimes also in the spaces between. The structure of the cortical parenchyma varies from homogeneous, with all the cells isodiametric, as in *P. lutea*, *P. senega*, and *P. polygama*, to heterogeneous, where the peripheral strata represent palisade, the inner ordinary parenchyma cells, and this structure occurs in the other species. A similar deviation was noticed in the leaves, where a ventral palisade tissue and a dorsal pneumatic occurs in *P. senega*, *P. lutea*, *P. sanguinea*, and *P. curtissii;* while in the remaining species the pneumatic tissue is inclosed by ventral and dorsal strata of palisade cells. The distribution of the stomata does not correspond altogether with these leaf structures, for *P. senega* is the only species showing a truly dorsiventral structure as to chlorenchyma and stomata; while in all the other species the stomata occur on both faces, whether the chlorenchyma shows a bifacial or centric structure, as just described. The mechanical tissue, stereome, is almost entirely absent from the leaves, but in the stem it forms a more or less closed sheath (pericycle) interspersed with thin-walled parenchyma surrounding the stele. In several species the pericycle is composed of two or three strata: the peripheral stereomatic, the inner parenchymatic. The increase in thickness of the stele depends on an interfascicular cambium, and not upon any activity on the part of the parenchymatic portion of the pericycle.

Summary

According to the material of North American species of *Polygala* here examined, it would seem that some of the subsections of Orthopolygala proposed by CHODAT are too heterogeneous to be considered really natural. For instance Incarnatae, comprising types so distinct as *P. incarnata*, *P. cruciata*, *P. mariana*, *P. sanguinea*, etc., are characterized by "stylus ovario longior, filiformis," which certainly applies only to *P. incarnata*. Moreover, the floral structure of this species (figs. 15–18) is very distinct from that of the others. The structure of Decurrentes *P. lutea:* "sepala cum pedicellis concrescentia i.e. pedicelli alati" was not to be found in the material here examined. The combination of *P. senega* and *P. polygama* into one subsection seems strange, when we compare the style structure and the different habit of these species, notably the cleistogamic flowers of the latter.

These four species, *P. incarnata*, *P. lutea*, *P. senega*, and *P. polygama*, are so distinct from all the others that they may be classified as monotypic subsections. With reference to *P. lutea*, the perennial habit by means of a persisting rosette of leaves would represent one good subsectional character; the floral structure a second, because of the development of a carpophore; and the wings, persisting for some time after the fruit has dropped, represent characters of greater importance than simply specific. The characters exhibited by the internal structure are merely specific, for instance, the centric leaf structure and the cortical parenchyma being homogeneous in some species and heterogeneous in others; as well as the occurrence of oil ducts in some species and its total absence from others. The peculiar secretions which VESQUE found in the chlorenchyma of several Brazilian species may be referable to oil, which CHODAT observed in the leaves of many South American species, but contained in lysigenous ducts. The crystalline appearance of the secretion found by

VESQUE was evidently due to the preparation of dried material. In the living plants examined the oil was perfectly normal, and sometimes quite abundant. But while CHODAT never observed these oil ducts in the stem of any of the South American species, they are nevertheless well represented, and very regularly indeed by some of the North American, as here described. The occurrence of secretions in *Polygala*, in species of limited geographic distribution in North and South America, thus represents an analogy to *Viola*, of which several species peculiar to Chile and eastern North America contain secretions in the roots, stems, and leaves.

Finally, with reference to epharmonic characters, according to my observations *Polygala* shows only a very few that may be identified as such. This is especially true of *P. senega* from woods and dense thickets, where the leaves show a strictly dorsiventral structure, while in the other species the stomata are distributed over both faces of the blade, whether the structure of the chlorenchyma is centric or dorsiventral. And this varied structure of the chlorenchyma accompanied by the constant distribution of the stomata is exemplified by species inhabiting more or less dry or moist sandy soil in the open, and very frequently associated with each other. While the leaves are destitute of mechanical tissue, such is present in the stems of all the species examined, but only in the form of a pericycle, and of a single stratum. The presence of oil ducts may hardly represent an epharmonic character, since it is not connected with any particular environment.

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DEVELOPMENT OF NORMAL AND DIVERGENT PLASTID TYPES IN ZEA MAYS

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(WITH PLATES X–XII)

Introduction

The transmission of plastid abnormalities has been shown to be conditioned, both by chromosome-borne genes, and by certain elements in the maternal gamete as yet unidentified. This second type of inheritance is thus far unique in that it alone appears to be non-Mendelian. The fact that, with the exception of *Pelargonium zonale*, the male gametes are unable to influence these maternally inherited deficiencies has been accepted by many workers as presumptive evidence that the material basis of this inheritance is located in the cytoplasm. While the exisiting genetic data permit an interpretation which would assign a nuclear basis for this inheritance, as DEMEREC (6) has pointed out, the fact that all known cases of maternal inheritance are concerned with plastid abnormalities strengthens the hypothesis that there is a cytoplasmic basis for their transmission.

The chloroplasts themselves would furnish a ready-made vehicle for this transmission, if it could be shown that they originated only through the division of pre-existing plastids which persist through all stages of the sporophyte and of the female gametophyte. The existence of such self-perpetuating chloroplasts can easily be demonstrated in certain algae and archegoniatae. In the higher plants, however, it can be shown that no such conspicuous cell organs as chloroplasts exist in certain stages in the life cycle. They must either originate *de novo* or develop from some self-perpetuating primordia. Such development from the primary meristem to fully differentiated tissue has frequently been traced in a number of species in both living and fixed material. No general discussion or review of the literature will be essayed here, as this has been done in some of the more recent papers, as those of EMBERGER (7), RANDOLPH (14), KASSMANN (8), KIRBY (9), etc.

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In any study of abnormal plastid types certain questions naturally arise. In what stage of the development can the abnormality first be recognized? Is the deficiency due to arrested development or to the degeneration of either partially or fully developed plastids? Is it possible to distinguish the primordia of abnormal types from those of the normal, and thus to trace directly the evidence of abnormality through the life cycle?

Zea mays has many advantages for an investigation of this kind. Numerous types of chlorophyll deficiency are known, whose specific conditioning genes have been located. These range from complete albinism to types which appear quite normal in the mature plant. Many different kinds of variegations are included, in some of which both normal and abnormal chloroplasts exist in the same plant. There are in addition three known cases of maternally inherited deficiencies.

The present paper contains a description of the normal plastid development, supplementary to a previously published account (16), and of the development of two albino stocks, a maternally inherited deficiency, a yellow-green lethal, an aurea, an argentea, and an ex-pallescent-virescent stock.

This investigation of plastid primordia is limited to fixed material, although mature and developing plastids were examined in living cells. While none of the fixatives used preserved the exact form of these primordia, their fixation images had been previously checked with living tissue (ZIRKLE 16), and thus in general it was possible to allow for certain known artifacts. There were, however, certain cases of unexplained variations in the fixation images. While it is possible that these may be due to an erratic behavior of an otherwise dependable fixative, the evidence indicates, as will be pointed out later, that they are caused by variations in the tissue fixed. The study of fixed primordia is, in a way, a necessary evil. Their small size and their index of refraction make it extremely difficult to differentiate them from ergastic material in uncleared cells. Their reaction to vital stains is highly non-specific, and, as RANDOLPH (14) reported, of little value in their study. At present their identification by their highly specific reactions to fixation is more certain (Kirby 9, Zirkle 17, 18), as well as by their clear-cut staining properties after being properly fixed and mordanted. Of course, under these conditions failure to find differences between those primordia which develop into normal plastids and those which develop into abnormal ones is in no way conclusive.

As the investigation was limited to plastids and their primordia, no attempts were made to preserve the various mitotic figures. The most satisfactory fixing fluid for this purpose was a modification of Erliki's potassium bichromate copper sulphate mixture. Erliki's fluid unmodified did not penetrate well. The leaves have such an impervious waxy cuticle that they could be fixed only by drawing the fluid up through the vascular bundles. The substitution of ammonium bichromate for the potassium bichromate overcame the difficulties of penetration but caused the outer layers of cells to be badly over-fixed. The most successful proportions for the fixative were:

$K_2Cr_2O_7$	1.25 gm.
$(NH_4)_2Cr_2O_7$	1.25 gm.
$CuSO_4$	1.00 gm.
H_2O	100.00 gm.

This mixture gave essentially the same image to both outer and inner layers. The fixation images of various other fluids will be described later. The only stain employed was Haidenhain's haematoxylin.

For the sake of definiteness, certain terms will be used in a strictly limited sense. The term mitochondria will include all rod-shaped, thread-shaped, or granular cytoplasmic inclusions preserved by bichromates on the basic side of the range pH 4.2–5.2 (the exact point depending upon the specific cation), and destroyed by those on the acid side of this range and by mixtures of bichromates and acetates. The term plastid will include only those cell organs which contain starch or chlorophyll.

NORMAL PLASTID DEVELOPMENT

In the growing point of the root the plastid primordia are mitochondria. Their shape and size are constant with a given fixative, but they vary greatly when fixed with different fluids. When the active fixing agent is formaldehyde they are thread-shaped; the same is true when they are fixed with ammonium bichromate. When fixed with potassium bichromate, copper bichromate, etc., they are rod-shaped. When the alkaline earths furnish the cation of the bichromate fixative, they tend to be granular or to form chains of granules. Lithium bichromate fixes them as distinct spheroids. While there is a slight variation of the size and shape of the mitochondria within a single cell, it is nowhere great and there is nothing to indicate two distinct types. There is no grading off in size to the border-line of visibility. The mitochondria elongate and divide transversely; and if any originate *de novo*, they must appear suddenly fully formed. There is a constant difference in the mitochondria in the different cells of this region regardless of the fixative used. They are larger and heavier in the epidermis and cortex and slenderer in the procambial cells and in the central cylinder. Constant as this difference is, however, it may be due entirely to an uncontrolled factor in the fixation. The fluid which first reaches and fixes the inner cells is more dilute than that which fixes those on the periphery, and considering how markedly the mitochondria react to slight differences in the fixation, this variation in size may be more apparent than real.

Slightly farther from the tip in the region of elongation the mitochondria are much more stable, fixing generally as rods; although with the bichromates of the alkaline earths they still tend to fix as chains of granules. With lithium bichromate, however, they are rods; and therefore by tracing a single line of cells back from the tip it is possible to trace the development of plastid-like bodies into distinct mitochondria, a reversal of the usual course of development.

In the region of differentiation two distinct sizes of mitochondria are found in each cell (fig. 5). These two sizes soon become quite distinct with no transitional stages between them. They are clearly fixed with the ammonium potassium bichromate mixture. The development of the larger ones can easily be traced until they are distinct plastids. The smaller apparently undergo no further change, and persist in the same form in cells which contain mature chloroplasts.

The presence of large starch grains has been reported frequently in the cells of the root cap. These grains are contained in mature plastids and occur in fully one-half of the cells of the cap. In the younger portion of the cap, the meristematic cells are in intimate contact with the apex of the root proper, and contain mitochondria of the same size and shape as those in the dermatogen, but no plastids. Between these two regions are cells which contain transitional stages. The whole development from primordium to mature plastid thus takes place within the range of a few contiguous cells, and all of the stages can be shown within a single microscopic field. The transitions from mitochondria to small plastids are shown in fig. 2.

The plastid primordia in the growing point of the stem are either mitochondria or minute plastids. As these two categories are but developmental stages of a single cell organ, the fact that either may occur in a given tissue indicates that plastid development is to a certain extent independent of the development of the plant as a whole. Just what growing conditions in the stem tip are prerequisite for the presence of either form is uncertain. There is no uncertainty, however, about the existence of either of these two stages. Fixed with ammonium potassium bichromate, the mitochondria are minute rods measuring 0.25 by 0.75 μ. The same fixative sometimes preserves the primordia as small spheres both in the stem tip and in the root tip (fig. 7). The plastids, small hollow spheres, can be identified by the occurrence of a minute starch grain 0.5 μ in diameter in their central vacuoles. These grains become very distinct when the balsam mounts are examined in polarized light.

The cells of the epicotyl immediately posterior to the convex growing point of the stem contain numerous small plastids. In the cortex four or five layers farther back the plastids are mature. Here therefore the development from primordia to plastids is completed within the range of as few cells as it is in the root cap. The epicotyl is apparently an important storage organ, as it contains numerous plastids all of which are full of starch. The one exception is the long procambial cell where the plastids do not develop as in the cortex but rather seem to degenerate. They become vacuolate, and their stainable substance collects in groups of two, three, or four peripheral granules (fig. 3). Later the plastid breaks down but the granules still tend to remain in groups of two, three, four, or five, four being the usual number.

If these granules can be classified as mitochondria, then they are the only mitochondria in these cells, since there are no other cytoplasmic inclusions with the fixing properties of such bodies. The ultimate fate of the granules is uncertain. It seems probable that they elongate into bodies which can be classified definitely as mitochondria; for the cells of young vascular bundles developing from these procambial cells are filled with mitochondria (fig. 4) which, farther back in older bundles (fig. 6), become transitional forms between mitochondria and plastids. Here the rods increase greatly in size and become vacuolate, each vacuole containing a single starch grain. The branch roots, which emerge from the lower nodes of the stem, and the prop roots, which grow down from the next higher nodes, contain mitochondria in their apical cells. The lineage of these cells can be traced back to cells near the growing point of the stem which contain numerous small plastids but no mitochondria. Thus it seems that the usual course of development from mitochondria to plastids is often reversed.

The primordia of chloroplasts in the chlorenchymous tissue of the embryonic leaves in young seedlings are minute plastids. They fix as small hollow spheres; and in normally growing green plants each contains a single starch grain in its central vacuole. There is little variation in size in these plastids within a single embryonic leaf until after the tip of the leaf has emerged, although there is a distinct increase in their size in the successive leaves from the stem growing point outward, until in the tip of the coleoptile they are almost mature. The coleoptile differs in some respects from the other leaves. While the cells in its tip contain plastids, those in its base contain mitochondria, much larger than the plastids in the inner leaves. In every leaf tip, as RANDOLPH reported (14), there are plastids, smaller in the younger leaves and larger in the older. The epidermal cells at the base of the embryonic leaves contain mitochondria.

Different cells in the mature leaf have very different kinds of plastids, as is shown in fig. 1. In the phloem the plastid primordia remain very small and fix as mitochondria. In the chlorenchyma between the bundles they remain relatively small, and are typical chloroplasts, seemingly solid bodies containing starch in their central vacuoles only when conditions have been favorable for photosynthesis. In the cells immediately surrounding the bundles the chloroplasts are much larger and are especially modified for the storage of starch. Each plastid in these cells contains a centrally located starch grain (fig. 1). When fixed with ammonium potassium bichromate, the pores leading into their central vacuoles (ZIRKLE 15) are enlarged and may easily be seen. When the plastid is so oriented that the flat surface is uppermost, the pores appear to be round, but when it is seen from the side they appear to be slits (fig. 1). This would indicate that the pores, somewhat concentrated on the flatter surfaces of the plastid, lead directly into the vacuole, yet do not point to the plastid's center.

MENDELIAN CHLOROPHYLL DEFICIENCIES

ALBINOS

DEMEREC (3) showed in his study of white seedlings that albinism could be caused by many different genes. There is nothing to indicate that the development of plastid primordia is alike in all these stocks. RANDOLPH (14) found plastid primordia in all of those cells in the albino which in normal seedlings contain green plastids, and even found green chloroplasts in the extreme tips of certain albino leaves. MILES (13), on the contrary, reported a complete absence of plastids in an albino stock, and figures no primordia. MILES' results are of little value, however, as he fixed the leaves he examined in a modification of Carnoy's fluid which destroys all plastid primordia. The investigations of two different albino stocks are recorded here.

In stock I, supplied by Dr. A. J. MANGELSDORF, the albinism is due to a single recessive gene which has not been located. It is a very vigorous, fast-growing stock. The albino seedlings normally reach a height of about 8 inches before they stop growing and die. When not exposed to direct sunlight they even reach a height of from 12 to 15 inches. No trace of green was ever observed in any of the albino seedlings.

Plastid development in the roots of these albinos is normal in every way, and indistinguishable from that in the green seedlings. The same is true for the plastid primordia in the growing point of the stem, and for the plastid development in the cortical cells of the epicotyl in young seedlings 3 or 4 inches high. It should be emphasized that the cells in the cortex are crowded with mature plastids, each of which is full of starch. The procambial cells are in every way normal, and the vascular bundles contain numerous transitional stages between mitochondria and plastids (fig. 6). In older seedlings which have reached the maximum height, however, the condition of the plastids in the now somewhat elongated epicotyl is quite different. Large starch-filled plastids still exist, but their numbers are so diminished that it appears as if their multiplication had not kept pace with the tissue's growth. In many of the cortical cells in the upper part of the epicotyl there are no plastids; instead one finds gigantic mitochondria 1 μ in diameter and from 20 to 50 μ in·length. It would seem that the seedling has lost the power of maturing plastids.

Plastid development in the leaves is very abnormal. The primordia in the leaves of the young seedling appear normal except for their lack of pigment. As growth progresses, however, they diverge more and more from the type. Distinct immature plastids 2 μ in diameter occur only along the edges of the leaf tips and in the terminal cells of the midrib. Some few cells in the bases of the leaves contain mitochondria. The greater part of the leaf, however, contains no recognizable cytoplasmic inclusions, although the cytoplasm of some of the cells is slightly granular. If any primordia are present they are very degenerate, and are not fixed by the fluids which ordinarily preserve mitochondria.

It is important to note that the state of the plastids in the albino seedlings is not due merely to their failure to complete development, but has an additional cause in a degeneration which ensues after they reach certain developmental stages. Except for the absence of pigments, the plastids appear entirely normal in the very young seedlings, and some leucoplasts actually mature in the epicotyl. In the older seedlings the only cells in the leaves which contain recognizable plastids are those in the tips which were formed either in the embryo or when the seedling was very young. This deficiency is due to the presence of a single homozygous recessive gene. The mother plant was of course heterozygous for this gene and had normal appearing green plastids. If the genes of the mother plant influence the plastid development in the embryo, or help to catalyze some substance necessary for complete plastid development which is stored in the seed, there is a ready explanation for the early development and later degeneration of plastids in the homozygous recessive albino seedlings. The stoppage of development and degeneration wou'd then be coincident with the exhaustion of this substance.

It has generally been assumed that the death of albino seedlings is due to starvation; unable to synthesize carbohydrates they can live only until the food stored in the seed is exhausted. It is interesting to note that death first occurs in the leaves where there is no stored food, yet the whole seedling dies while the epicotyl still contains some stored starch. Obviously death is not due entirely to a lack of carbohydrates.

The albino seedlings are just as phototropic as the normal green type. A tray containing both green and albino plants was illuminated from one side. Both types of seedlings grew toward the light, and no difference in kind or amount could be noted in the reactions of the two groups.

Stock II was supplied by Dr. W. H. EYSTER and described by him as often producing green stripes and being sometimes viable. Under growing conditions in the greenhouse of the Bussey Institution this was a very feeble stock, the seedlings rarely reaching a height of 3 inches. They showed no trace of any green tissue and were apparently pure albinos, although it would perhaps be more accurate to describe them as extreme cases of virescence. Their plastid development was normal except for a complete lack of color. Even just before death there was apparently an adequate supply of minute plastids in all of the cells in the mesophyll (fig. 9).

GREEN-YELLOW LETHAL

This stock was supplied by Dr. W. H. EYSTER. The population obtained consisted of approximately three parts normal plants and one part of greenish yellow seedlings, which latter seedlings died after reaching the height of about 6 inches. As has been found in every case thus far, no differences could be observed in the plastid primordia between the deficient and the green seedlings. The small plastids in the young leaves seemed normal. When they reached a fourth of the mature size, however, further development ceased and they began to degenerate (fig. 8). This degeneration sometimes precedes the death of the plant by several days. The plastids become ragged, tend to fragment, and very darkly staining granules appear about them in the cytoplasm. The cells are full of débris when death occurs.

AUREA

This stock was also furnished by Dr. W. H. EYSTER, who has not as yet published the genetic data.[2] The deficiency is due to a gene in chromosome I. Seedlings showing the character are a golden yellow, and are much lighter in color than the greenish yellow lethal just described. EYSTER reports that the aurea seedlings have a full complement of xanthophyll, carotin, and chlorophyll b, but little or no chlorophyll a. The seedlings are very vigorous and grow rapidly, and, when they reach the height of 18 inches the tips of their leaves begin to turn green. The mature plants are green and are but little lighter than those which are normal.

The plastids, except for pigment content, seem completely normal throughout the entire plant. They are especially large, well developed, and numerous in the yellow leaves. When the seedlings are 6 inches high, the stage where the green-yellow lethals die, the diameter of their plastids is three times that of the latter. It should be emphasized here that the death of certain lethal types, which are identified by their chlorophyll deficiency, may be caused by factors much less conspicuous than the lack of pigment. The two types just described are cases in point. The stock which had the greater amount of pigment but defective plastid development was lethal; the stock which had less pigment but well developed plastids was viable.

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[2] At the time of reading proof these data have been published: EYSTER, W. H., Five new genes in chromosome I in maize. Zeitschr. Indukt. Abstam. Vererb. **49**: 105–130. 1929.

ARGENTEA

This chlorophyll pattern, due to a gene in chromosome I, was also supplied by Dr. W. H. EYSTER (genetic data not published). The seedlings, which at first appear to be a type of virescent, vary greatly in their amount of pigment, and likewise show great variation in rate of growth, the darker seedlings growing more rapidly. The first two leaves of the average 6-inch seedling are white except for a green midrib and border. The third and fourth leaves show more chlorophyll. In the half-grown plant the first four leaves and the tip of the fifth show many white or light green stripes, which, alternating with darker tissue, give the whole a silvery sheen. The other leaves are usually normal, although in extreme cases the whole plant will have very minute light green stripes.

The influence of this gene makes itself felt during a single limited period in the growth of the seedling. It does not influence the first foliage tissue, as is shown by the midrib and the tips of the first leaves being normal green. It delays or inhibits plastid development in the first four leaves and the tip of the fifth. When the bases of the fifth and later leaves develop, the gene usually ceases to influence plastid development, as this tissue appears normal.

Plastid primordia are entirely normal in this type. Their development in certain leaf tissue is inhibited or prevented from going beyond a certain point. In the fine light green or white stripes the plastids rarely reach over one-third of their diameter in the darker stripes. This is especially evident in the cells which surround the vascular bundles (fig. 13).

EXPALLESCENT-VIRESCENT

This aberrant type is segregated from a stock supplied by Dr. A. J. MANGELSDORF. It is due to a single recessive gene which has not as yet been located. When these seedlings first emerge from the ground they cannot be distinguished from their normal sibs. After 3 or 4 days, however, they lose most of their color. The first leaf becomes pale green while the second, third, and fourth leaves become white except for green midribs and borders. There is a great variation in the amount of pigment in the various plants at this stage. Some of the lighter plants die, while the darker ones grow only about half as fast as a normal seedling from the same ear. Gradually these plants acquire chlorophyll. The first leaf becomes a bright green while the next few leaves acquire green stripes. Leaves subsequent to the fifth are essentially green, although they contain several white stripes averaging 0.25 inch in width. The mature plants contain these stripes, although there is great variation as to their number and distribution.

The primordia here, as in all other types, are normal, as are also the earlier stages of development. There succeeds a period in which development is checked and apparently reversed for a time. Normal development then proceeds to maturity. Many plastid types can be obtained from the different leaves of the same plant and from the same leaf at different times. Fig. 14 shows half-grown plastids in the third leaf when it is developing green stripes. The granular cytoplasm shown is often found at this stage. It is worthy of record that in every abnormal type here investigated the deficiencies do not appear until after the seedling has been growing for a time independently.

MATERNAL INHERITANCE CHLOROPHYLL DEFICIENCY

This stock was sent in the spring of 1926 by Dr. W. H. EYSTER, who has determined but has not yet published on the genetic behavior. It is apparently a third instance of maternal inheritance in *Zea*. Three types of seedlings were produced by the segregating seeds: light green seedlings, which die when they reach the height of about 6 inches; seedlings with longitudinal light and dark green stripes, most of which mature and produce all three types of offspring; and normally green seedlings, which produce only normal offspring. This maternally inherited abnormality is quite different from the one described by RANDOLPH (14) and ANDERSON (1), and corresponds in all essential details with that described by DEMEREC (6).

The striped seedlings show a great variation in the amount of light and dark tissue which they possess, ranging from the entirely light seedlings to those entirely normal. The lighter variegated ones live but a little while after their completely light green sibs die. In the later stages of development their growth is very abnormal. Stem growth stops and the leaves, without increasing in width, elongate greatly and become pendent. It is no unusual sight to see one of these seedlings 6 inches in height with leaves over a foot long. Before death occurs, the lighter portions of these leaves become much paler than in the seedlings, which possess no dark tissue. When the light and dark areas are about equal the seedling is practically as vigorous as those normally green.

In the very young plant, when the light and dark stripes can first be recognized, the light area is the color of the pale green seedlings, and the color of the dark area is normal. As growth progresses the difference in color of the two regions becomes more pronounced. The darker regions become more intense while the light regions fade. All stages in stripe formation can be observed in plants as they reach the tasseling stage. The stripes on the older leaves at the bottom of the plant may be dark green and cream colored. The stripes in the tips of the upper leaves may be light and dark green. If these stripes be traced toward the leaf bases, it can be seen that their shades approach each other until on reaching the bases their colors become identical, and no stripes whatever are visible. The shade of the leaf base is that of normal young chlorenchymous tissue.

No cytological difference can be observed in the plastid primordia which develop into the normal and divergent plastid types. The mitochondria in the root tips and the plastids and mitochondria in the stem growing point and epicotyl are the same in all three types of seedlings.

The differences in plastid development must be traced in the leaves. It is obvious that the difference between the plastids in the light and dark areas depends upon the age of the tissue. In that region of very young leaves where the stripes can just be recognized, no difference whatever can be observed in the individual plastids of the two regions. In both living and frozen sections the plastids are the same size and color. In fact the two regions are much more distinct macroscopically than in microscopic sections. The earliest distinguishable differences in shade are due to the relative number of plastids and their arrangement in the cells (fig. 10). This difference is particularly clear in the chlorenchymous cells contiguous to the vascular bundles. In the darker stripes these cells contain numerous plastids arranged around their peripheries; in the lighter stripes they contain few plastids. In tissue a little older, differences can be observed in the plastids themselves. Their development becomes retarded in the lighter stripes, so that these are somewhat smaller and lighter than in the darker portions of the leaf. A cytological difference in the types can here be demonstrated. When the tissue is fixed with ammonium potassium bichromate, stained in haematoxylin and destained, the aberrant plastids lose their color before the normal ones. This is shown in fig. 11. In still older tissue the plastid differences have increased. In the lighter areas the plastids have degenerated, while in the darker they reach mature size. The degenerating plastids fragment and the fragments disintegrate. When the light stripe is cream colored its cells contain nothing recognizable as plastids (fig. 12).

It is of distinct theoretical importance to determine whether there are but two kinds of chloroplasts in the striped seedlings or whether transitional stages exist on the border-line between the stripes. It is also of importance to know whether more than one kind of plastid can be recognized within a single cell. This problem is complicated by the fact that different types of plastids exist in normal leaves. An investigation of very young tissue does not determine the point, for the variation in plastid size from cell to cell exists in unvariegated leaves. In older tissue the answer is plain. There are two separate and distinct types of plastids and no transitional stages. The borders of the stripes, however, are seldom clear-cut and precise. The plastids in the cells surrounding the vascular bundles are apparently quite independent of those in the mesophyll between the bundles. Frequently the cells contiguous to the bundles have normal plastids, while the mesophyll cells which surround them contain no recognizable plastids (fig. 12); on the other hand, the latter cells may contain normal plastids in regions of the leaf while those which surround the vascular bundles contain the aberrant type (fig. 11). At no time were both kinds of plastids observed within a single cell, and frequently cells containing normal plastids occurred well into the lighter regions of the leaf (figs. 11, 12). This localization of a single plastid type to each cell is in keeping with what ANDERSSON (2) found in *Adiantum cuneatum*. The questions as to whether or not these two kinds of plastids are conditioned by two distinct categories of primordia, which are, however, morphologically alike, and whether two types of primordia exist in a single egg, cannot at present be answered.

Summary

Plastid primordia in the root tips of *Zea mays* are mitochondria. In the root cap their development into mature plastids can be traced within a single microscopic field. In the region of differentiation of the root proper two distinct types of mitochondria occur, the larger of which develops into plastids while the smaller apparently undergoes no further change. In the stem growing point the primordia are generally minute plastids, that is, hollow spheroids containing starch grains 0.5 μ in diameter in the central vacuoles, although occasionally they are mitochondria. Back from the growing point of the stem in the epicotyl, the primordia quickly develop into plastids. In the procambial cells plastid-like bodies can be observed to fragment, and there is some evidence that these fragments develop into mitochondria. In the vascular bundles many transitional stages can be observed in the course of development of mitochondria into plastids. In the leaves the primordia develop into mitochondria in the phloem, into large starch-containing plastids with prominent pores in the cells immediately surrounding the vascular bundles, and into smaller, apparently denser plastids in the chlorenchymous cells between the bundles. In all aberrant types investigated, the plastid primordia were normal so far as could be observed, the divergent types being due to (1) a delayed development, (2) a stoppage of the development, and (3) a stoppage of development followed by degeneration. In the first albino stock investigated the mode of development in the young plastids suggested an influence exerted by the genetic constitution of the mother plant. A disintegration of the partly developed plastids in the leaves of this stock occurred in all except the earliest-formed cells of the foliage tissue, that is, the tips of the first, second, and third leaves. The second albino stock was evidently an extreme case of virescence. All of the mesophyll tissue of the leaves contained minute plastids. Both albino stocks died while starch was still stored in the epicotyl, indicating that starvation was not the primary cause of death.

The divergent plastids in the maternal inheritance stock developed as in normal tissue until they were about half-grown. The first signs of abnormality were not in the plastids themselves but in their number and arrangement within the cells. In tissue of half-grown leaves, a distinct size difference could be observed between the plastids in the light and the dark stripes. In old tissue the plastids in the lighter areas degenerated and disappeared. Mature leaves showed but two kinds of plastids, and contained no intermediate forms on the border-line of the two regions. In the green-yellow lethal stock the plastids degenerated after reaching one-fourth the normal diameter. In "aurea" plastid development was quite normal except for the pigment content. In both the "argentea" and the expallescent-virescent stock the abnormality was due to a checking of plastid development early in the life of the seedling, and to the later normal plastid growth. In general, the abnormal period was between the formation of the tip of the first leaf and the formation of the base of the fifth leaf. Both stocks were very variable.

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EXPLANATION OF PLATES X–XII

The photographs were made from slides stained with Haidenhain's haematoxylin. No counter stain was used. All were fixed with the ammonium potassium bichromate modification of Erliki's fixative.

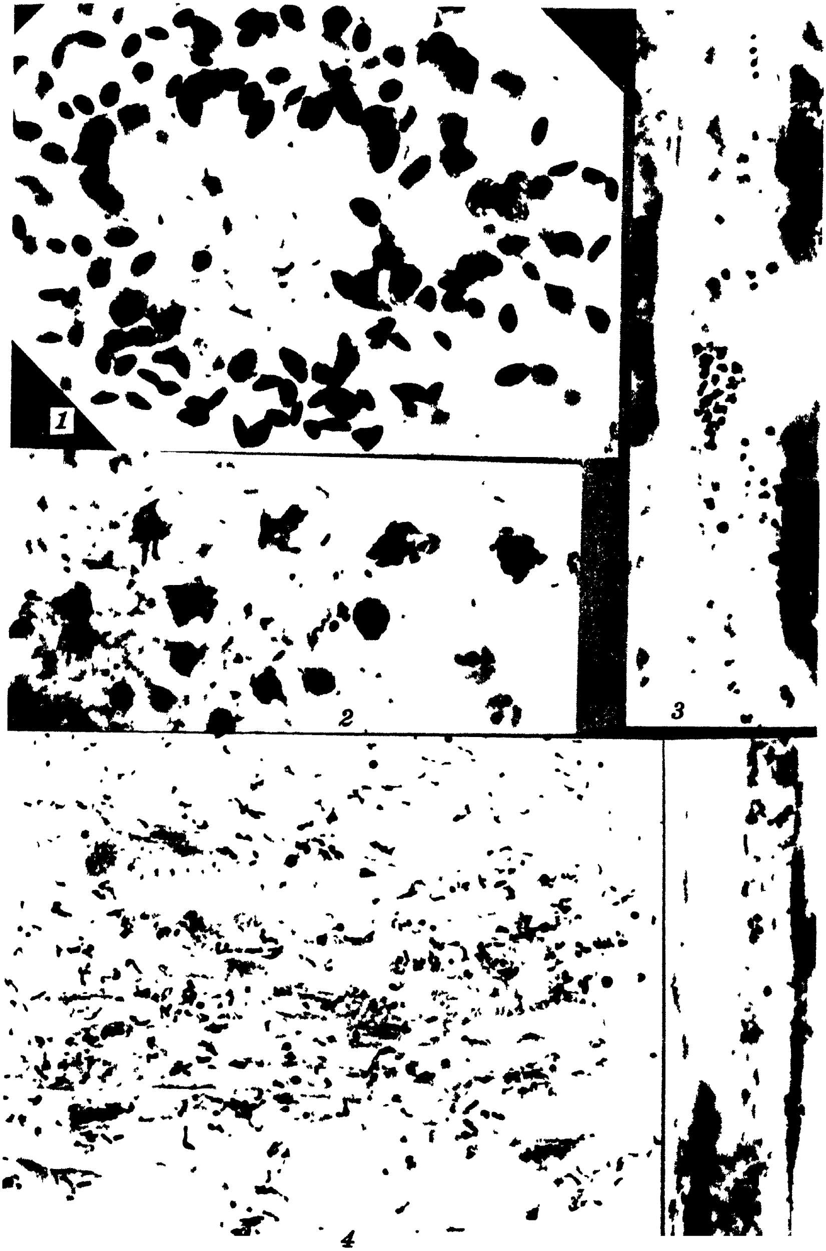
PLATE X

FIG. 1.—Cross-section of leaf of normal green plant; three end products of development of plastid primordia are shown: (1) mitochondria in vascular bundle, (2) large starch-containing plastids with prominent pores in cells surrounding bundle, and (3) smaller typical plastids in mesophyll; ×1000.

FIG. 2.—Longitudinal section of root cap showing mitochondria (on left) developing into plastids (on right); ×750.

FIG. 3.—Longitudinal section of procambial cell in epicotyl showing plastids breaking up into granules; ×1500.

FIG. 4.—Longitudinal section of vascular bundle in epicotyl showing mitochondria and transitional stages; ×400.



ZIRKLE on ZEA MAYS

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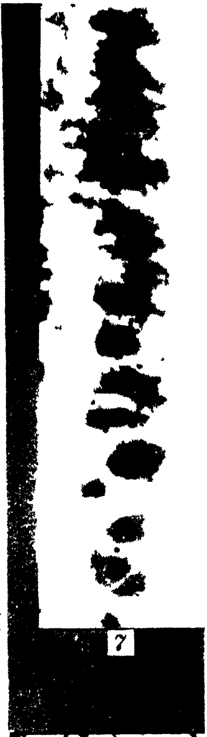
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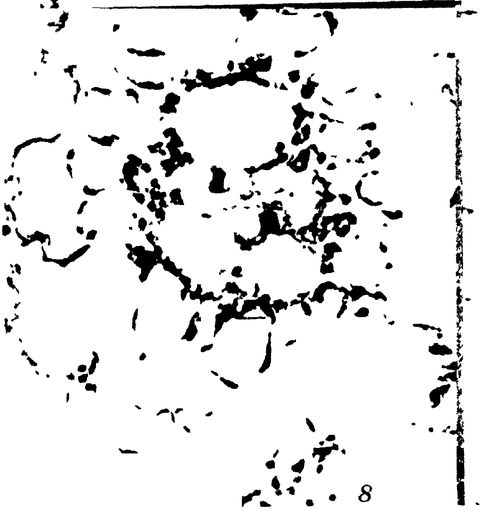
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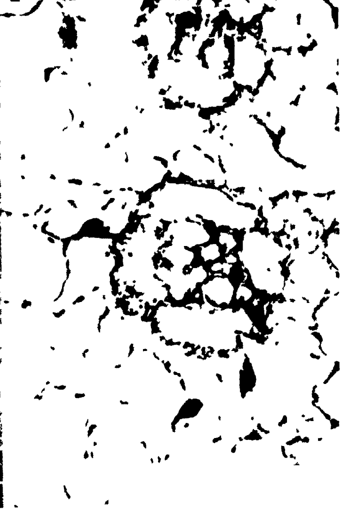

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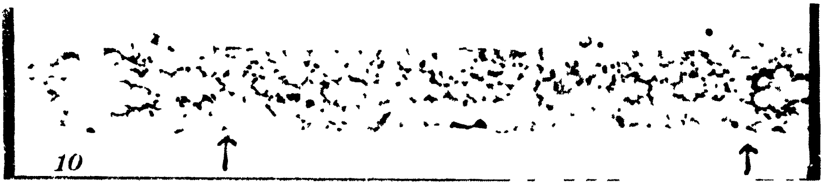

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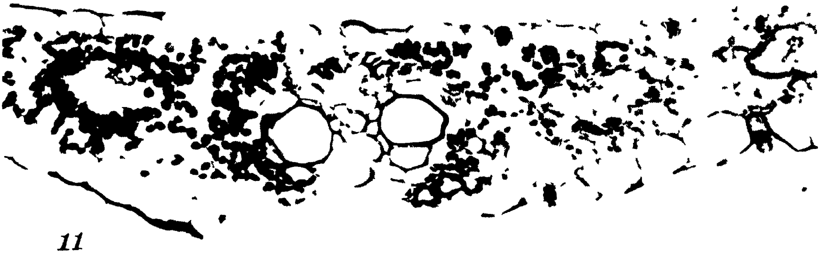

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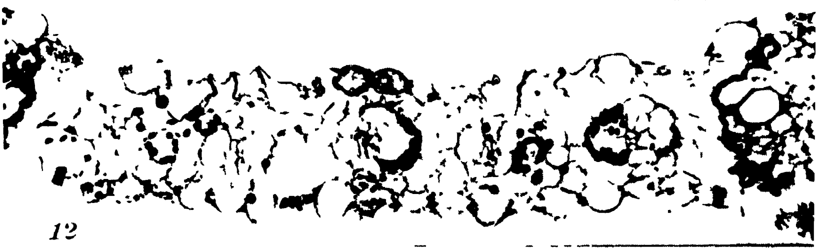

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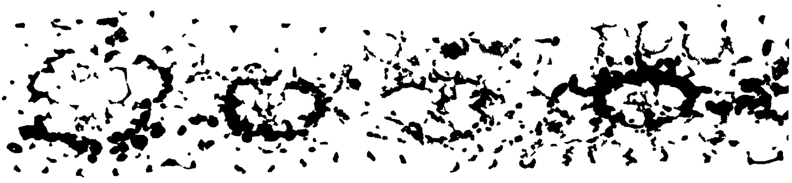

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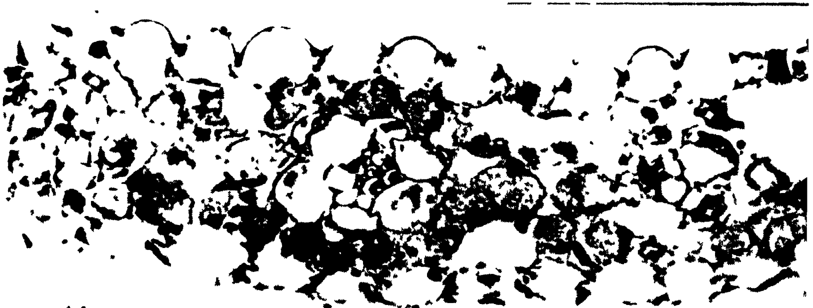

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ZIRKLE on ZEA MAYS

FIG. 5.—Longitudinal section of root in region of differentiation, showing two sizes of mitochondria; ×800.

PLATE XI

FIG. 6.—Same tissue as fig. 4, older stage; transitional stages plainer here; ×800.

FIG. 7.—Epidermal cells near tip of root showing occasional plastid-like form assumed by mitochondria; ×800.

FIG. 8.—Cross-section of leaf of yellow-green lethal stock; ×250.

FIG. 9.—Cross-section of leaf of second albino stock; probably an extreme case of virescence; ×250.

PLATE XII

FIG. 10.—Cross-section of leaf showing earliest recognizable difference between dark and light areas in maternal inheritance stock; arrows mark limits of white stripe; ×125.

FIG. 11.—Cross-section of older leaf of same stock, showing difference in size of two types of plastids and difference in ability of types to retain stain; ×175.

FIG. 12.—Cross-section of still older leaf, showing disappearance of plastids in lighter region and fuller development of plastids in certain cells; ×175.

FIG. 13.—Cross-section of "argentea" leaf, showing small plastids in minute light green stripe; ×175.

FIG. 14.—Cross-section of leaf in expallescent-virescent plant, showing half-grown plastids and granular cytoplasm; ×200.

MULTIPLE CONES IN ZAMIA FLORIDANA

FRANCES GRACE SMITH

(WITH FOURTEEN FIGURES)

Since a summary of the views concerning the stem development of cycads was included in a former paper (6), it is necessary here only to mention the results of investigations made available since that date. MATTE (5), in the introduction to his monograph, includes a historical sketch of the subject, but in his own investigations seems not to have worked upon the base of the peduncle nor to have considered its origin. CHRYSLER (3), in his study of *Microcycas calocoma* from Cuba, states that cone domes are present; that the cones are single and terminal.

CHAMBERLAIN (1), studying *Macrozamia moorei* from South Africa, finds that ovulate cones, two to eight in number, and staminate cones from twenty to one hundred and three on an unbranched plant, are borne clearly in a lateral position. He considers the stem monopodial in its development, and without cone domes, all cones coming from the axils of leaves, as in Cycadeoidea. In an unpublished paper upon *Bowenia serrulata*, Mrs. ALICE BAILEY shows cones borne on slender branches, the cone arising in the axil of a leaf. It was not possible from the meager material on hand to ascertain whether or not cone domes were present. CHAMBERLAIN'S (2) clear exposition of the presence of cone domes in all stems with a terminal strobilus furnishes a supplementary criterion for a determination of the stem type in the different cycad species. This relation, as well as the average number of cones produced, is shown in table I.

While the species of *Dioon*, *Ceratozamia*, *Microcycas*, *Stangeria*, and *Bowenia* have a single cone as a rule, the species of *Zamia* frequently bear more than one cone and these close to the tip of the stem. When the strobilus is single the method of development of the trunk is clearly sympodial, and the development might in certain cases be rapid enough to bring to maturity several cones in the same season. On the other hand, so many of these cases were found and the number of staminate cones to a plant was so large that, in view of the situation in *Macrozamia* and *Encephalartos*, it was thought worth while to examine *Zamia* a little more thoroughly as to the origin and direction of the bundles, so that the former statement of the presence of a sympodium might be corrected or emphasized.

TABLE I

SPECIES	OVULATE CONES	STAMINATE CONES	CONE DOMES
Dioon	Terminal, single as a rule	Terminal, single as a rule	Present in both
Ceratozamia	Terminal, single as a rule	Terminal, single as a rule	Present in both
Microcycas	Terminal, single as a rule	Terminal, single as a rule	Present in both
Zamia	Terminal, frequently more than 1, sometimes 6 or 7	Terminal, frequently more than 1, 10 not uncommon	Present in both
Cycas	Not compact, sporophylls making a crown	Terminal, single	Absent in ovulate, present in staminate plant
Bowenia	On slender branches, single	On slender branches, single	?
Encephalartos	Lateral, often more than 1, 3–5 common	Lateral, many	Absent in both
Stangeria	Terminal, single as a rule	Terminal, single, occasionally 2 or 3	Present in both
Macrozamia	Lateral, 2–8	Lateral, many, up to 100	Absent in both

Methods

Of the many plants of *Zamia floridana*, collected and sent from Miami, Florida, fifteen were used for this study, having from two to ten cones each. Four plants bore ovulate cones, two to four on each crown; eleven bore staminate cones, three to ten each. These are about the usual proportions, for the exceptions to the single cone condition are more numerous among staminate plants, and the number of cones is larger.

The plants were cut 3–4 inches below the crown (the leaf blades were trimmed before they were sent), and the petioles and the upper part of the strobili removed, leaving the peduncles and enough of the sporophylls to determine whether they were megasporangia or microsporangia, and whether they were old or young. A diagram of each plant, looking onto the crown, was made to show the point of origin and the comparative size of the cones. Then serial sections were made, cut longitudinally 2–5 cm. thick, killed in alcohol and formalin-acetic acid, washed, dehydrated, and cleared in xylol with as careful a transition between grades of alcohol and xylol as in small specimens. The longer the immersion in xylol the clearer the course of the bundles became. In some cases carbon disulphide was added to the xylol. The sections were studied over an electric light bulb and drawn with a camera lucida.

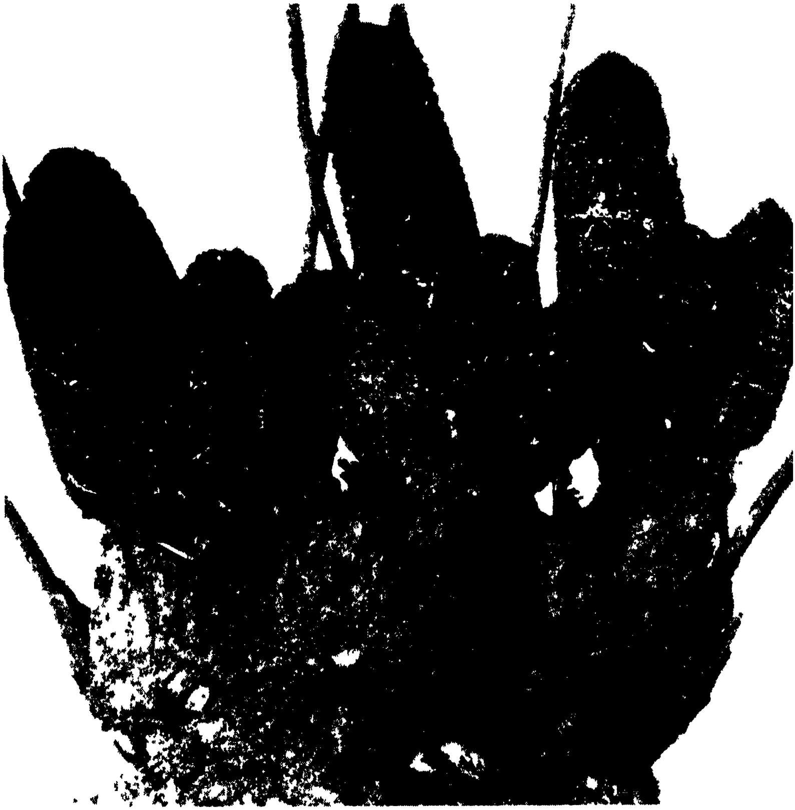

Fig. 1.*—Crown of plant with ten staminate cones, leaf blades and some leaves removed.

The cones were all of this year's growth; in most cases even the peduncles of last year's cones were so shriveled that they were hard to recognize. When removing the petioles from the plants before sectioning, it was noted that frequently the compact head of cones resolved itself into a much branched one (figs. 1, 2). Whether this was due altogether to external causes or not it is impossible to say, but fire often sweeps through the open pine forest in which *Zamia* makes a part of the undergrowth, and partially destroys the foliage and probably some of the trunk. Sometimes the land has been roughly cultivated and the plants cut up; sometimes "the practice is to dig only the upper portion of the plant, leaving the tap root which soon produces another harvest for the starch mill." This accounts for the wide branching of *Zamia* in fig. 3, my collector writes. Several branch tips showed young cones when received in August and September.

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* All figures are of *Zamia floridana*.

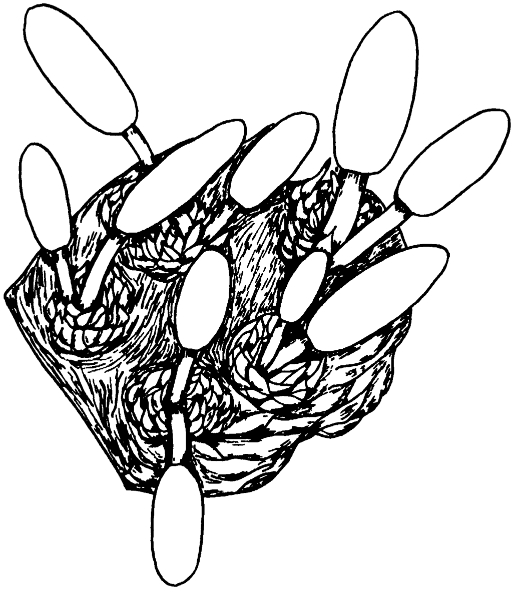


FIG. 2.—Same plant as fig. 1 with scale leaf removed, showing five buds developed from crown; each bud has two cones.

Of the plants studied, one ovulate and four staminate showed good examples of typical sympodial growth. In the other plants this was not so simple, for at one point at least, in each specimen, there was a definite forking of the bundles (that is, a lateral direction with reference to the terminal bud). Figs. 4 and 5 show the simple sympodium in an ovulate and a staminate plant respectively, while fig. 8 (staminate) shows a sympodium in each branch. In fig. 5 the

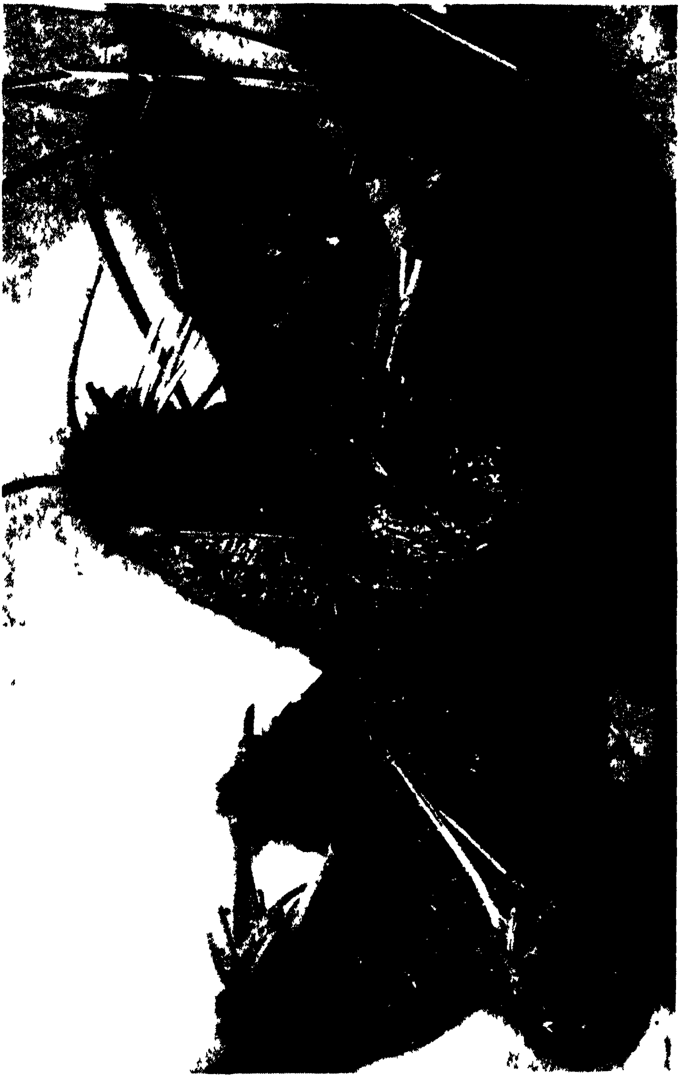
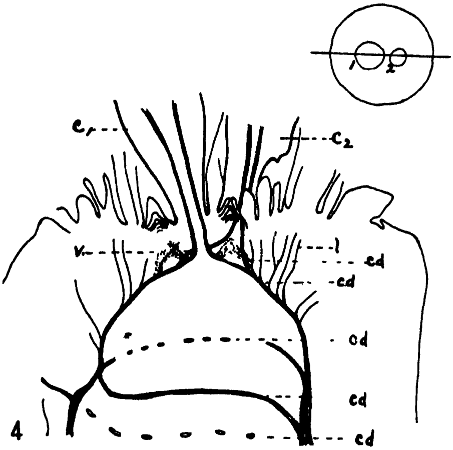


FIG. 3.—Photograph of plant developed after injury to crown



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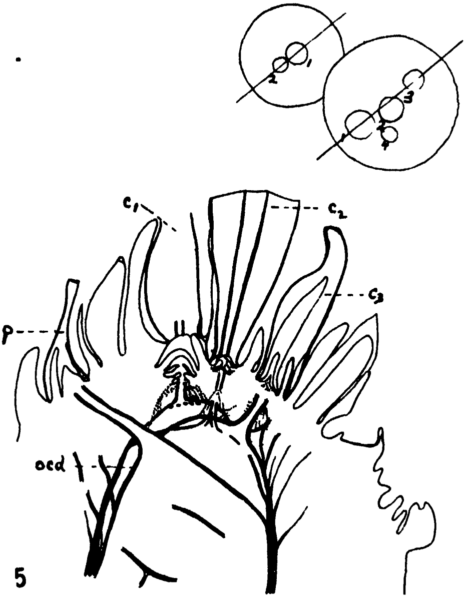

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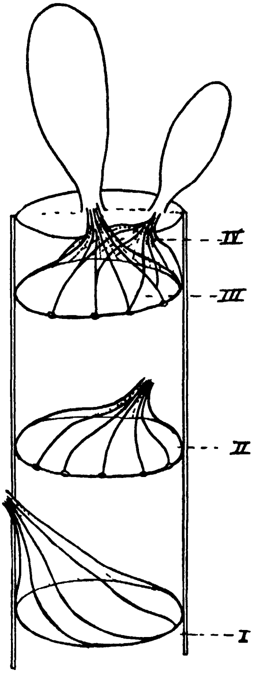

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FIGS. 4–6.—Fig. 4, longitudinal section of crown with two ovulate cones and showing a good sympodium (cf. fig. 6), lowest cone dome (*cd*) cut transversely across bundles, as shown in II of fig. 6; next cone lengthwise, as in I of fig. 6; the third transversely again, as in II; the fourth like III of fig. 6, the fifth like IV of fig. 6; *l*, leaf trace, *v*, vegetative point. Fig. 5, longitudinal section of staminate-coned plant: *op*, old peduncle, not visible in crown diagram; *ocd*, old cone bundles; cone numbers agree with those of diagram (note order of formation of cone domes and attachment of bundles of each to preceding cone dome). Fig. 6, diagram of cone dome formation: I, II, III, IV, different views of cone domes.

shriveled peduncle on the left does not show in the plan of the crown; the three next older cone domes are indicated and three vegetative points are developing.

In the cleared sections these can be distinguished by the greater

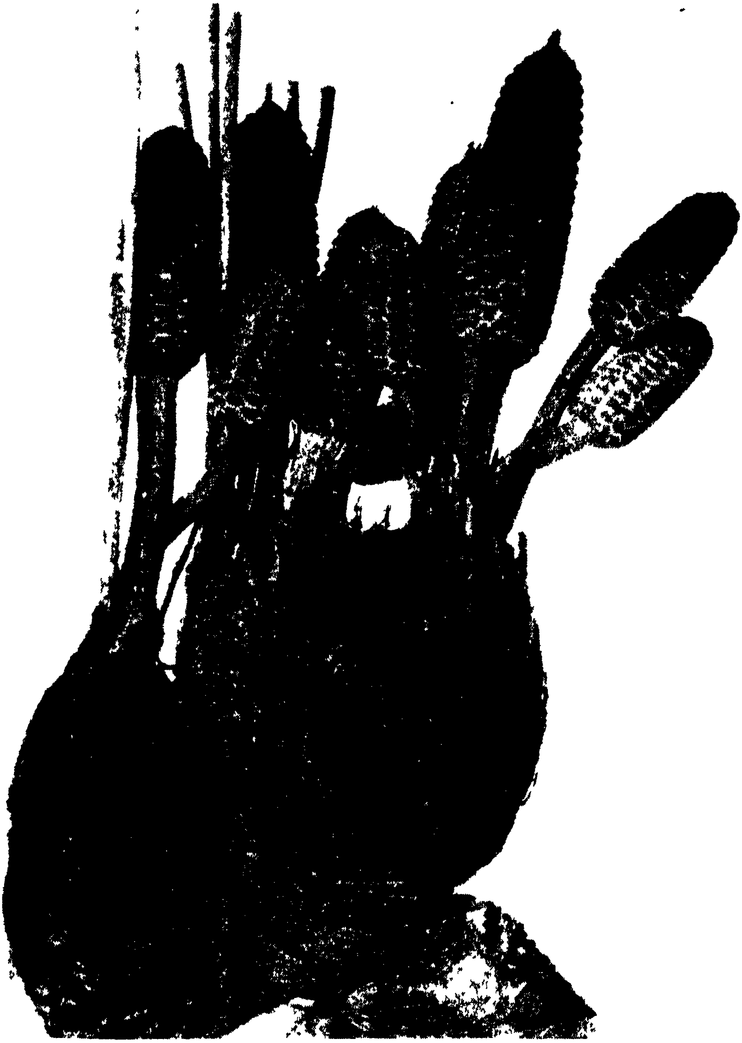


FIG. 7.—Plant with ten staminate cones, two of which are on bud below level of crown.

density of the cells (lesser transparency), and, as indicated in the drawing, they frequently appear close to the converging bundles of the cone next older, rather deeply placed in the tissue. No attempt was made to trace the development of the meristem before the appearance of the bundles in the cone, but their course could be followed back until they fused with older bundles, making a one-sided cap around the cone dome preceding (figs. 4, 5). In many sections the fine veins running to the leaves could be clearly distinguished. In fig. 4 the lowest cone dome was cut transversely across the bundles, the separate bundles showing cross-sections of xylem and phloem. In the next cone dome they were cut lengthwise; the third was transverse; and the fourth showed the origin of the bundles of the oldest cone to be seen on the plant and the arches of this dome made by the bundles as they curve to enter the peduncle. Fig. 6 illustrates this diagrammatically. Fig. 7 (a photograph) and fig. 8 (the longitudinal section) illustrate a plant with ten staminate cones having in each arm or fork a succession of cone domes, and so close together

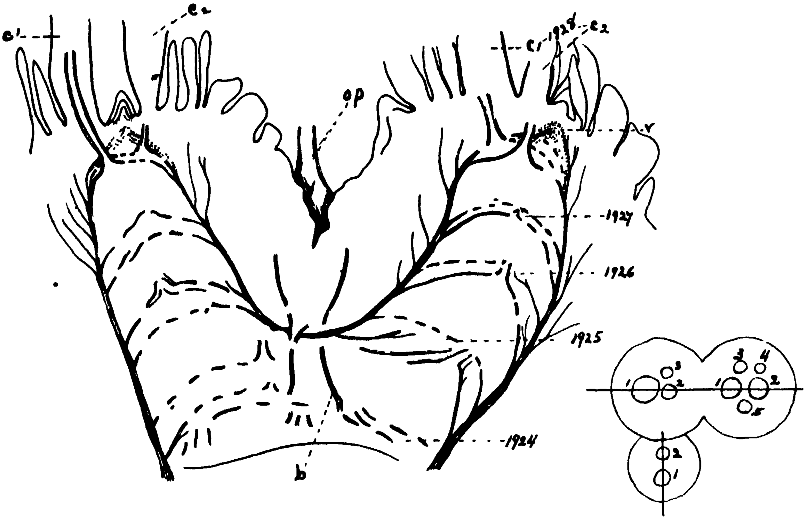


Fig. 8.—Longitudinal section of same plant as in fig. 7 with two branches represented; *b*, bundles to an old peduncle (*op*) which does not show in crown diagram below; two branches develop sympodially with two cones each year as shown by cone domes.

have shown. There were two cones on the bud and eight on the trunk.

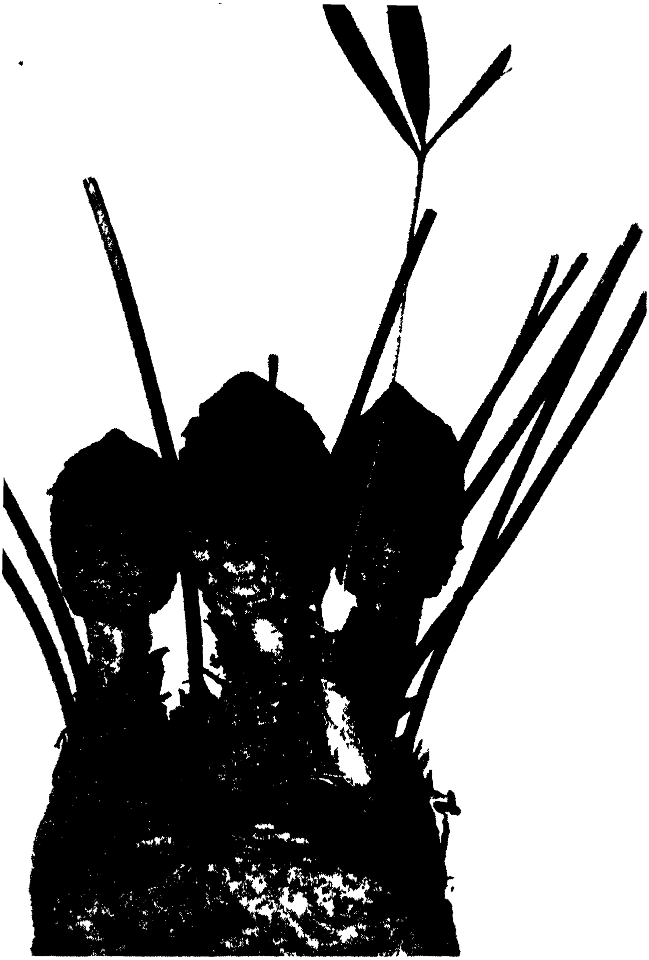
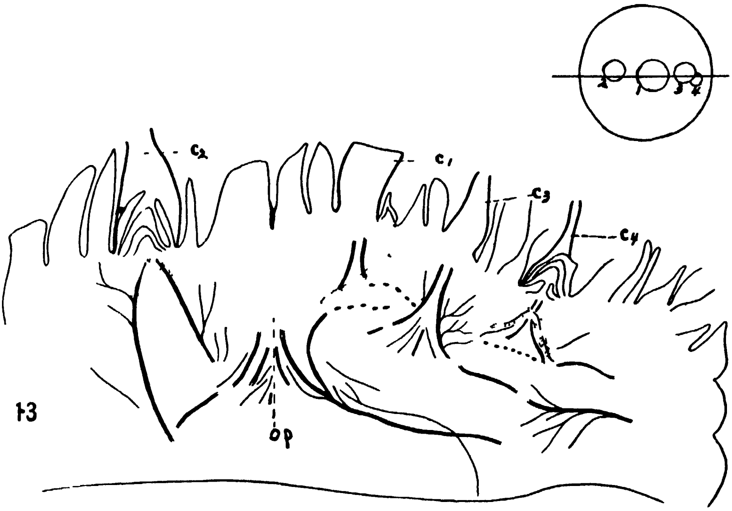


FIG. 12.—Ovulate plant with very broad crown

That adventitious buds develop may be the explanation of the first photograph and the figure accompanying it, which show ten cones. The longitudinal sections could disclose only three of these adventitious buds at a time, but they show the connections of the cone bundles with the main stelar bundles, and no cone domes appear close to the tips of the branches. COULTER and CHRYSLER (4) record two types of "regeneration" in *Zamia:* (1) adventitious bud-



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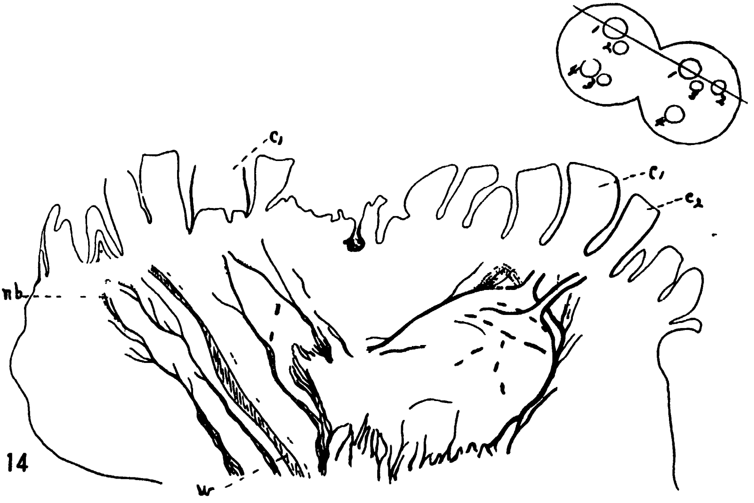

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FIGS. 13, 14—Fig. 13, longitudinal sections of same plant sympodially developed on right, *op*, old cone bundles, at left of these another cone and growing point developing as lateral offset. Fig. 14, longitudinal section of plant with eight cones, deeply stained streak at left (*w*) made by cut in tissue, bundles at right and left of this crack seem to arise directly from main stelar tissues; *nb*, new bud.

ding, and (2) the actual restoration of lost parts. They find "these new shoots arise most frequently from the vascular part of the central cylinder but also from the peripheral part of the wounded surface of the cortex," i.e. the wound phellogen. In the plant illustrated in fig. 11 there is no evidence of wounding, unless a very one-sided development of the crown may be it. The three pairs of cones are distinctly traced back to the main stelar bundles of the trunk. There was no trace of cone domes in this part, but in the shoot which is very vigorous, on the left in fig. 11, two cones already have formed and the middle pair will show them next season. The very broad crown gives a diagonal section of the stelar region, but the tip is toward the left, so that the other buds are almost lateral in relation to it (fig. 11). In fig. 14, referred to before, the development of a new bud is close to what looks like a long and deep cut in the tissue, but the connections of the buds are with the vascular system at a distance from the vegetative points of the buds, and there is no sign of any relation to the neighboring wound phellogen. MISS STOPES (7) records the development of buds in *Cycas* from leaf bases. It is evident that here we have only the most common type of shoot formation from the vascular part of the central cylinder.

Summary

Multiple cones in *Zamia floridana* may have three methods of development:

1. They may be traced back to a sympodium which shows a rapid formation of cone domes one after another. This is most common.
2. Sympodial development of cone domes is found in certain branches, but in them the forking of the bundle system begins near the base of a terminal cone, which was not turned to one side but remained erect as shown by the remnant of its peduncle.
3. Adventitious buds appear in the cortical tissue closely connected with the stelar system of the trunk, and these buds continue their development like typical stems.

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SWARMING OF DINOFLAGELLATES IN DELAWARE BAY, NEW JERSEY[1]

G. W. MARTIN AND THURLOW C. NELSON

(WITH FOUR FIGURES)

The marked discoloration of extensive areas of ocean water, as well as quiet bodies of fresh water, known as red water, bloody water, milky water, and by similar terms, has been noted since ancient times. The vivid description of the turning of the waters of the Nile into blood, found in the seventh chapter of Exodus, probably refers to something of the sort. Numerous references, including citations from STRABO, TACITUS, and other classical writers, and from a number of navigators of the sixteenth to the early nineteenth centuries, are given by DARESTE (3). The causes to which these appearances have been attributed are various, and sometimes very curious; but for the past hundred years it has been recognized that they are due to the presence in the water of dense swarms of minute and commonly microscopic organisms. These may be blue-green algae, copepods, diatoms, or members of various other groups, but the most striking instances are due to dinoflagellates. CHARLES DARWIN (4) saw extensive areas of such water off the coast of Chile in 1832, and describes the organisms causing certain of them in such a way as to leave no doubt that they were dinoflagellates, although he does not name them. ALLMAN (1) reported an outbreak of brown water in freshwater ponds of the Dublin parks, due to a dinoflagellate which he called *Peridinea uberrima*. CARTER (2) noted such water near Bombay, and attributed it to a form which he named *Peridinium sanguineum*. WHITELEGGE (10) describes an extensive outbreak of red water in New South Wales, due to *Glenodinium rubrum*, which caused great devastation among oysters and other marine animals not able to escape from the area. In his opinion the devastation was due to suffocation caused by the exhaustion of the oxygen in the water, complicated, after the first attack, by the fouling of the water from the decomposition of the dead bodies. NISHI-

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[1] Contribution no. 16, New Jersey Oyster Investigation Laboratory. Paper of the Journal Series, New Jersey Agricultural Experiment Station.

KAWA (9) reports an outbreak of red water in Japan produced by *Gonyaulax polygramma*. This did no damage, but earlier outbreaks in the same locality were reported by local residents to have been highly destructive to pearl oysters, fishes, and crustaceans. HIRASAKA (5) reports another case in Japan due to *Gymnodinium sanguineum*, which did no damage, and cites an earlier outbreak reported by OKAMURA (also cited by KOFOID and SWEZY 7) in which some injury to fish was observed. Perhaps the most striking examples are those reported by KOFOID (6) from coastal waters of California, due to *Gonyaulax polyhedra*, where in some cases the discoloration was many miles in extent, and was accompanied by great devastation among the bottom-living animals. KOFOID and SWEZY mention an outbreak of yellow water, due to *Gymnodinium flavum*, and accompanied by a great display of luminescence.

A similar condition seems to be of rather common occurrence in parts of Delaware Bay, New Jersey, especially in the area lying to the east and northeast of Deadman Shoal, Maurice River Cove. Through the kindness of Mr. J. RICHARDS NELSON, in charge of oyster investigations of the New Jersey Agricultural Experiment Station and Board of Shell Fisheries in Maurice River Cove, we have been able to observe a number of striking exhibitions of red and brown water during the late summer of 1928.

On July 30, at 8:00 A.M., with the tide at approximately one hour ebb, a belt of brown water was seen along the shore below Dias Creek, Cape May County. This brown belt extended approximately 7 meters out from the shore to a depth of 2 meters, where it met in a very definite sharp line the gray-brown water of the bay. With but slight variation in width, it stretched up and down the beach as far as the eye could distinguish it. The sky was clear, and a gentle south-southwest wind stirred the surface. At low tide the broad flats of sand and mud ran bare, leaving sloughs with water a few centimeters deep. This water, exposed to the direct rays of a hot sun, continued to show the same brown color, resembling weak tea.

Examination of the water showed it to be swarming with a small, yellowish green, fusiform dinoflagellate, which belonged to an apparently hitherto undescribed species, and which has since been described (8) under the name *Amphidinium fusiforme*. The organisms were exceedingly active for a few minutes under the microscope, finally coming to rest and almost immediately disintegrating.

As the flood tide came in over the flats, the brown water became mixed with the incoming waters of high turbidity and the brown color disappeared. Oysters which had been exposed during low tide were opened after the water had risen half a meter over them and their stomach contents immediately examined. These were found to consist of a veritable soup of disintegrating dinoflagellates, chiefly *Amphidinium fusiforme*, with numerous specimens of the much larger *Gymnodinium splendens*.

On August 2 at 10:00 A.M., red water was observed over the oyster grounds of Inner Deadman Shoal, in water approximately 6 meters deep. The tide was high, beginning ebb, with a hot sun and a light west wind. Three belts of color were seen, each 3–5 meters broad and extending for several hundred meters. In hue the water varied from that of weak cocoa to a distinct blood red, as though great quantities of blood were being mixed with the sea water. The water in the belts had a temperature of 25.0° C., pH 8.2 with Cresol red, and a specific gravity of 1.01614 (corrected to 17.5° C.). Water just outside the belts had the same temperature, but a pH of 8.15 and specific gravity of 1.01610. A sample of the red water observed under the microscope showed enormous numbers of *Amphidinium fusiforme*, together with numerous other dinoflagellates. In watching the belts from the deck of a boat, it seemed as though a given spot varied in intensity, thinning out to the hue of weak cocoa and then quickly increasing to a blood red color.

On August 24 at 11:40 A.M., with the tide 2 hours' ebb, a belt of red water some 20 meters wide and several hundred meters long was encountered over the oyster beds of Inner Deep Water. The sun was shining brightly, and short choppy waves 1–2 feet high were running against the tide, under a moderate south wind. As the waves passed through the belt they became partially flattened out, as though in an oil slick.

On August 30, about midday of a hot clear day, a large number of patches and belts of red water were observed. The tide was about half ebb, the sea fairly calm, and the sunlight intense. The temperature of the water varied from 28.6 to 28.8° C. The patches varied in color from chocolate brown to deep blood red, and in area from a few square rods to many acres, being mostly rather long and narrow. One such patch could be observed from the roof of a cabin, perhaps 8 feet above the surface of the water, to extend for at least a mile in either direction. The line of demarkation between the red water and the clear green normal water was sharp and distinct, as the accompanying photograph (fig. 1), snapped with an ordinary pocket camera without a filter, demonstrates. Examination of plankton samples from various patches of red water showed that in most cases *Amphidinium fusiforme* was the dominant species present. Associated with it in large numbers was a *Gymnodinium* agreeing closely with *G. uberrimum* (Allman) K. & S. as described by KOFOID and SWEZY (7). Reference to ALLMAN'S original paper (1), however, raises a question as to whether ALLMAN'S *Peridinia uberrima* really is, as KOFOID and SWEZY claim, identical with the form they call *Gymnodinium uberrimum*. It seems better, therefore, not to attempt to apply the specific name at this time.[2] In a few samples the *Gymnodinium* was the dominant species. Associated with these two forms were numerous other dinoflagellates, particularly *Gymnodinium splendens*, *Polykrikos kofoidi*, and species of *Gonyaulax* and *Peridinium;* also diatoms, particularly *Sceletonema costatum* and species of *Coscinodiscus*, *Biddulphia*, and *Chaetoceros*.

The contrast between the yellow-green color of *Amphidinium fusiforme* and the yellow-brown color of the associated *Gymnodinium* (as seen under the microscope by transmitted light) on the one hand, and the red and reddish brown color of the patches of red water in which they occur on the other, is very significant, suggesting strongly that these densely packed masses of chlorophyll-containing cells make of the surface layers of the water essentially a nearly continuous suspension of chlorophyll, and that the reddish color is nothing other than the well-known reddish fluorescence of chlorophyll. This is borne out by the fact that the red shades predominate in the deeper water, while where it is shallow enough to permit light to be reflected from the bottom, through the organisms, the brownish color predominates.

What causes dinoflagellates to mass together in this way has

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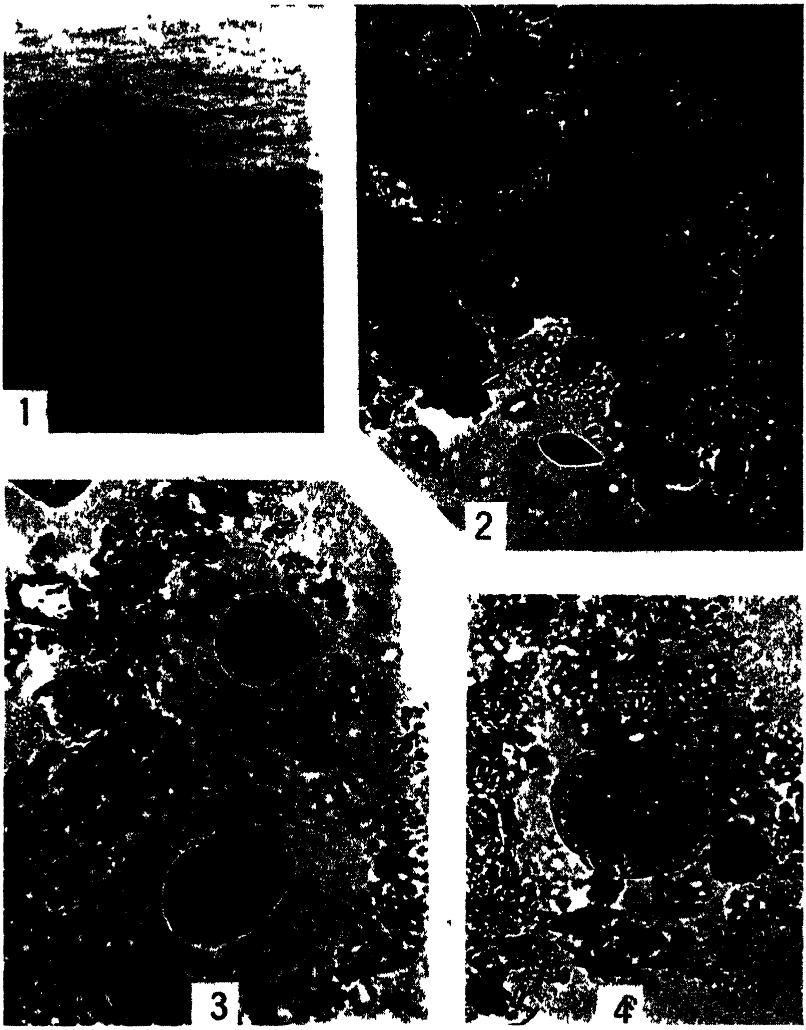
[2] Since this manuscript was submitted, the larger *Gymnodinium* referred to has been described (Univ. Ia. Studies Nat. Hist. 12:16. 1929) as *Gymnodinium subrufescens* Martin.



Figs 1–4.—Fig. 1, margin of patch of red water showing sharp delimitation of area, Maurice River Cove, August 30, 1928. Fig. 2,* photomicrograph of plankton from patch similar to that illustrated in fig. 1, and collected at same place and date, mass of *Amphidinium fusiforme*, together with colloidal detritus and a few diatoms, collected about a cell of *Gymnodinium;* killed in osmic acid, followed by chrom-acetic solution, then formalin, and stained in Trypan blue. Figs. 3, 4, cells of *Gymnodinium*, showing thick, hyaline gelatinous envelope, margin of which is outlined by clinging detritus, killed in saturated solution of bichloride of mercury, followed by formalin, and photographed unstained, Deadman Shoal, August 29, 1928.

* Figs. 2, 3, and 4 photographed at magnification of approximately 800 diameters and reduced one-fifth in reproduction.

never been explained, but observations made upon this occasion may have some bearing upon the matter. Red water plankton, in which the *Gymnodinium* predominated, when killed with a strong solution of iodine in potassium iodide and examined microscopically, showed each cell of the *Gymnodinium* to be surrounded by a gelatinous envelope about as thick as the diameter of the cell itself, so that the water in which they occurred must have been a nearly continuous mass of soft jelly. This gelatinous envelope is also visible in material killed with a saturated solution of bichloride of mercury, although it is not quite so apparent, since neither the jelly nor the cells are stained by this fixative (figs. 3, 4). It is less regularly seen in material killed by osmic acid and then transferred to chrom-acetic solution, and never in any of the other reagents used. Gelatinous envelopes are common among dinoflagellates when encysted, but not when active. The cells referred to in this connection were actively motile. A similar envelope has been noted occasionally surrounding other species of naked dinoflagellates in the active condition, but only when killed by the iodine or bichloride methods. In red water plankton in which *Amphidinium fusiforme* is the dominant species, the *Amphidinium* cells tend to cling together in clumps, but no gelatinous envelope can be demonstrated. In many of the clumps (although not in all), however, they may be seen to be clustered thickly about a cell of the *Gymnodinium* (fig. 2). This gelatinous envelope may well be a factor of importance in holding the organisms together, once they are massed by a favorable combination of light, water temperature, and tidal currents.

In no case was there any suggestion of injury to fish or other marine animals in connection with the swarming. On the contrary, MR. NELSON reports that it is just those areas in which red water is most frequently encountered that have been found by long experience to provide the best fattening grounds for oysters in the entire district. Experience elsewhere, as shown by the references here cited, suggests that these dense swarms may constitute a potential menace to shellfish and to other marine animals. Probably the relatively swift tide, and the comparatively small area occupied by the red water in Delaware Bay, render the possibility of damage to oysters in that region rather remote.

Summary

1. Instances of red water occurring in Delaware Bay and due to the swarming of *Amphidinium fusiforme* and other dinoflagellates are described.

2. It is suggested that the characteristic color of the water which gives the phenomenon its name is due to the reddish fluorescence of the chlorophyll present in such great quantities.

3. A factor tending to hold the cells together in dense masses, once they are brought together, may be the gelatinization of the outer envelope of the active cells, not heretofore noted in this connection.

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CURRENT LITERATURE

BOOK REVIEWS

Enzymes

Biological investigators will welcome the recent translation of WALDSCHMIDT-LEITZ's[1] book on enzymes. The author attempts to give a concise account rather than an exhaustive treatise of the main principles of enzyme chemistry, and the special characteristics of the most prominent enzymes. The viewpoint is essentially that of the WILLSTÄTTER school, and many of the results of WILLSTÄTTER and his co-workers, which have recently been published,[2] are summarized in the book. This is only natural, since WALDSCHMIDT-LEITZ is an active associate of WILLSTÄTTER. The book is not merely a summary of the results obtained by the WILLSTÄTTER school, however, but is intended as a general summary of enzyme literature. It is a good volume from which to get a review of the work of foreign investigators, especially the German workers. One feels that the work of English and American scientists has been neglected, although this is perhaps to be expected in a work of this kind. The translator has materially extended the original German text, the additions, however, being mainly according to the viewpoint of the WILLSTÄTTER school.

The book is divided into two main parts. The first is a general section, with chapters on development of the ferment concept, enzymes as colloids, enzymes as electrolytes, enzymic kinetics, enzymic reaction systems, enzymic specificity, and detailed procedures in preparative enzyme chemistry. One of the main contributions of WILLSTÄTTER and his co-workers has been the devising of methods of obtaining pure enzyme preparations. These methods are rather fully outlined. In the second section the main enzyme systems are treated. There are chapters on esterases, proteases and peptidases, aminoacylases, carbohydrases, catalases, peroxydases, oxydases, and fermentation enzymes. In the chapter on the aminoacylases, urease is treated, and SUMNER'S investigation which resulted in the isolation in the crystalline state of a protein of the globulin group, identical with urease, is referred to. It is interesting to note that the author states that the German workers, EULER and BRUNIUS, in their recent investigation have been unable to confirm SUMNER'S findings. SUMNER attributes their failure to a low urease content in the material used. It would seem that some such explanation for their failure must be true, for SUMNER'S work seems rather definitely to prove that the urease of the jack-bean and the crystalline protein isolated from the jack-bean are identical.

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[1] WALDSCHMIDT-LEITZ, ERNEST, Enzyme actions and properties. Transl. by ROBERT P. WALTON. pp. xv+255. New York: John Wiley & Sons. 1929.

[2] WILLSTÄTTER R., and coworkers, Untersuchungen über Enzyme. vols. 2. pp. xvi+1775. Berlin: Julius Springer. 1928.

One of the main values of the book is that it gives an authoritative modern viewpoint of enzyme chemistry. This is very much needed, for much of the older literature on enzymes is conflicting and therefore worthless for arriving at a true conception of the nature of enzymes and their action. For example, it is a common statement in the literature that some enzymes are given off first in the cell in an inactive state called "zymogen" or "proenzyme," but the author states that this viewpoint has not been proved. The enzyme merely appears to be in the inactive form because some condition for its activity, very commonly the pH, is not right. While WILLSTÄTTER has not perhaps been as successful in elucidating enzyme chemistry as he was in determining the chemical constitution of chlorophyll and other complex organic compounds, yet his contributions to our knowledge of enzymes have been many. A book that summarizes some of the results obtained by this master of organic chemistry and gives his viewpoint is especially to be welcomed.—S. V. EATON.

A new state flora

SCHAFFNER, who has seen many years of service at the Ohio State University, has produced a field manual of the flora of Ohio and adjacent territory.[3] The book is attractively bound, and, from its small dimensions, merits the name pocket manual. A formal and full phyletic system is introduced for the "complete classification into phyla, classes, subclasses, orders, and families." In many of the larger families and genera, condensed synopses are given. The numerous keys, based as they are upon floral groups unlike those of any other one area of similar size, afford many fresh viewpoints as to diagnostic characters, and will doubtless find use at the hands of students in neighboring states. The author acknowledges help from a long list of his past students who have studied special groups of the Ohio plants. The nomenclature follows the American Code, and is essentially in agreement with that of the second edition of Britton and Brown's *Illustrated flora*. A partial adaptation to GRAY's Manual (7th edition) is afforded by means of generic synonyms and occasional specific synonyms. These latter are far from complete (cf. *Prunus virginiana* and *P. nana*, p. 305; *Vaccinium angustifolium*, p. 391), leaving the work less useful for class work where a correlation with GRAY's Manual is desired. Furthermore, the number of common names for each species is reduced, in the interest of nomenclatural clarity, to one for each species. It remains to be seen whether a state flora will be generally recognized as the proper agency for the introduction of such an innovation, desirable as this might seem to some workers.

Many of the treatments differ from what might be expected. This appears due in some cases to the author's personal views, in others to his failure to accept the findings of various workers outside his own state. Thus (p. 357) the name *Quercus rubra* L., which really applies to a tree of the southern and southeastern United States, is retained for the red oaks (*Q. borealis* Michx.) of Ohio; (p. 501) *Xanthium americanum* Walt., a name of very dubious application, is permitted to displace *X. chinense* Mill., and *X. canadense* Mill. is erroneously cited as a synonym for *X. pennsylvanicum* Wallr.; (p. 520) the name *Bidens trichosperma* (Michx.) Britt. is retained despite the precedence of the name *B. coronata* (L.) Britt.; (p. 558) the old and outgrown theory of FRIES, KOCH, and certain others, of the oneness of all dandelion species is readopted, at least in part, making our red-seeded dandelion (*Taraxacum laevigatum* Willd.) and our brown-seeded species (*T. vulgare* Lam.) one and the same.

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[3] SCHAFFNER, JOHN H., Field manual of the flora of Ohio and adjacent territory. 16mo. pp. 638. Columbus, Ohio: R. G. Adams and Co. 1928.

Numerous typographical errors, especially in the spelling of scientific names, occur to mar the appearance of the book. One notes also various other errors, such, for example, as the omission of *Papyrius* from the main text and index although it is included on p. 583 in the key to Ohio woody plants. The reviewer is led to hope that a second edition may be preceded by a careful revision in these respects.—E. E. SHERFF.

Evolution of genetics

More than success has been attained by OEHLKERS in his recent volume.[4] He set out to give a sketch of the development of plant genetics during the past fifteen years; he has in fact retraced the significant steps in this field since the rediscovery of Mendel's laws. This little book is more than a sketch of the past, it orients critically, and looks into the probable future of genetics. This comprehensive presentation is especially useful and timely, because the enormous and rapid development of the subject has tended to isolate the specific problems and investigators, so that they and their problems get out of touch, not only with the rest of the field of genetics, but even more seriously with the larger botanical problems. The author points out that genetics stands at the turning point. The first period, the analytical, which was characterized by the study of the delineation and transmission of factors, is approaching the end of its domination in genetics, even though much more analytical work remains to be done; and the second period, the synthetic, is already under way. The latter will concentrate more and more on the relationship of genetics to developmental physiology and morphology. Granted the genes, there still remains the greater problem of what lies between transmission of the genes to the zygote and their expression in all the various stages of the developing and mature organism. Another important question is the precise significance of the genes in evolution. In going back more than fifteen years the author is no more than just to his subject matter, for the problems which have become acute were sensed in the early days of genetics, but were neglected or ignored in the progress of factorial analysis. Some of these problems are stated as chapter and paragraph headings in the book.

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[4] OEHLKERS, FR., Erblichkeitsforschung an Pflanzen, ein Abriss ihrer Entwicklung in den letzten 15 Jahren. Wiss. Forschungsberichte. 18. pp. 203. Dresden and Leipzig: Theodor Steinkopff. 1927. $14.50.

The subject matter is discussed under two main headings, hybridization and mutation. Under the first heading, the general part takes up (1) Mendelian rules, (2) cycles of development (life cycles), (3) nucleus and heredity, (4) and (5) chromosomes and heredity, (6) protoplasm and heredity; while the special part takes up (1) sterility and lethality, (2) sexuality, and (3) significance of hybridization in species formation. Under the second heading the following topics are discussed: (1) concept of mutation, (2) factor mutants, and (3) genom mutants. Each chapter is concluded with a bibliography. The volume is well and clearly written.—G. K. K. LINK.

NOTES FOR STUDENTS

Chlorophyll.—The most significant experimental contribution that has recently appeared is a paper by EMERSON[5] on the chlorophyll content and rate of photosynthesis. The methods of WARBURG have been used in this investigation, and the results are as valuable as those obtained by him in his work on the respiration enzyme. Optical methods were used for estimating the amount of chlorophyll, while WARBURG's methods were used to measure the amount of oxygen liberated. By a careful study of the "photochemical reaction" and the "Blackman reaction" (the ordinary chemical reactions involved in plant growth), EMERSON has concluded that chlorophyll plays some other part in living processes in the plant, besides its rôle in the photochemical reaction.—F. M. SCHERTZ.

Chromosome numbers in Oenotheraceae.—*Circaea lutitiana*, *C. alpina*, and *C. intermedia* have been recently studied.[6] In all of these the diploid number is twenty-two and the haploid eleven. The rather plump chromosomes of the first-named species sometimes show a cross constriction. The other two species have more slender chromosomes, and the constriction is often so deep as to give the appearance of division of one chromosome into two. The author, quoting observations of recent writers, gives the haploid chromosome number for other genera of Oenotheraceae as follows: *Oenothera* 7, *Eucharidium* 7, *Godetia* 7 (and 9), *Jussieua* 8, *Clarkia* 9, *Lopezia* 11, *Fuchsia* 11 (and 14), *Epilobium* 18, and *Chamaenerion* 18.—FRANCIS RAMALEY.

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[5] EMERSON, R., Chlorophyll content and rate of photosynthesis Proc Nat. Acad. Sci 15:281–284. 1929.

[6] UDDLING, ÅKE, Die Chromosomenzahlen von drei Circaea-Arten. Hereditas 12:294–296. 1929.

VOLUME LXXXVIII NUMBER 3

THE

BOTANICAL GAZETTE

November 1929

POTASSIUM DEFICIENCY IN SUGAR CANE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 395

CONSTANCE ENDICOTT HARTT

(WITH FOURTEEN FIGURES)

Potassium has been included in lists of essential elements since 1804, when the necessity of potassium salts for terrestrial plants was established by DE SAUSSURE (6). Since then attention has been focused upon the rôle of potassium, but its exact function has not yet been definitely determined. A good general review of the literature dealing with potassium is given by RUSSELL (29). The work reported here is an attempt: (1) to obtain gradations in growth correlated with gradations in the potassium supplied in the nutrient solutions; and (2) to secure, if possible, correlations with physiological processes, especially enzyme activity.

The literature contains many references to experiments with prepared enzymes, for example, Taka-diastase and Merck's diastase of malt. Such material has not been prepared from plants grown in a potassium-free nutrient solution, and it is generally made from seedlings which are too young to show potassium starvation phenomena. To determine the effect of potassium upon enzymes it is really necessary to use material prepared from plants starved for potassium, compared with controls. This is the method of DOBY and HIBBARD (7), and has been used in the present investigation.

Methods

I. CULTURAL

Cuttings of Cheribon (Louisiana Purple) sugar cane, obtained from Dr. E. W. BRANDES of the United States Department of Agriculture, were used in this investigation. Similar experiments with wheat, buckwheat, and soy bean will be reported in a later paper.

Twelve healthy cuttings of sugar cane with roots already starting were covered with sterilized quartz sand in a flat on October 25, 1927. They were watered well with distilled water and placed in a warm greenhouse. The first sprout appeared in three days and was quickly followed by others. They were allowed to become well rooted before being moved. On November 22 the plants were cut from the seed pieces with a sterilized knife and planted in 2-gallon glazed earthenware crocks, one plant in each crock. About 2 inches of seed piece remained on each plant. The plants were watered with distilled water for three days and then, since no mortality occurred due to the transplanting, watering with the solutions was begun. Artificial light was used during the winter to supplement the daylight (two 200 watt bulbs with reflectors over approximately the upper third of the bulbs).

The quartz sand was sterilized in 20 per cent hydrochloric acid followed by thorough washing with distilled water. The solutions were made up on the basis of SHIVE's (30) best, which is a 3-salt solution with an osmotic concentration of 1.75 atmospheres. Plants were grown with four different amounts of potassium, equivalent amounts of sodium being added to replace the potassium and maintain the solutions at equal osmotic pressure. Iron was added as ferric phosphate according to the directions of LIVINGSTON (18). The following nutrient solutions were prepared from volume molecular stock solutions, each made up to a liter:

Solution I (SHIVE's best) control: 18 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2 \cdot 4\ H_2O$ +15 cc. $MgSO_4 \cdot 7\ H_2O$.

Solution II: 18 cc. $NaH_2PO_4 \cdot H_2O$+5.2 cc. $Ca(NO_3)_2 \cdot 4\ H_2O$+15 cc. $MgSO_4 \cdot 7\ H_2O$.

Solution III: 17.9 cc. $NaH_2PO_4 \cdot H_2O$+0.1 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2 \cdot 4\ H_2O$+15 cc. $MgSO_4 \cdot 7\ H_2O$.

Solution IV: 17 cc. $NaH_2PO_4 \cdot H_2O$+1 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2 \cdot 4\ H_2O$ +15 cc. $MgSO_4 \cdot 7\ H_2O$.

Three plants were grown with solution I and four with each of the others. Throughout this report numerals I–IV will designate the plants grown in these respective solutions. The plants were watered with these solutions as needed. Drainage was maintained by bent Pyrex tubes inserted through the opening in the bottom of each crock, which operated as siphons and could be closed with pinch clamps.

II. COLLECTION AND PREPARATION

One plant of each group was collected early in the afternoon of April 14 and taken immediately to the laboratory, where measurements of size, weight, etc., were made. The tops were removed, ground rapidly in a Russwin mill, and the pulp thoroughly mixed. Duplicate portions were immediately weighed and set aside in watch glasses with ground glass edges, held together with clips, for moisture determinations. Duplicate portions for sugar determinations were weighed in Erlenmeyer flasks containing a little calcium carbonate and boiled in 95 per cent alcohol. The rest of the material was placed in evaporating dishes, covered with several layers of cheesecloth and evaporated to dryness with an electric fan. It was then stored in desiccators. The roots were dried without being ground.

The second collection was made early in the afternoon of June 2. The same procedure was followed except that the leaf blades were separated from the sheaths, the latter being included with the stems.

III. QUANTITATIVE DETERMINATIONS OF ENZYME ACTIVITY

The diastase activity was determined quantitatively by the rate of disappearance of starch as shown by the color with IKI.

Quantitative determinations of the activity of invertase were made by measuring the increase, during 24 hours, in the ability to reduce Fehling's solution. The reducing action was determined by the method of MUNSON and WALKER (23). The BERTRAND method (3), titration with potassium permanganate, was used for estimating the amount of copper oxide formed in the Fehling's solution. The relative activity of peptase was estimated by comparing the amounts of color liberated from carmine fibrin by equal amounts of material in a given time and under the same conditions. The carmine fibrin was prepared according to the directions given by HAWK (11), and is a substrate suggested by GRÜTZNER (8).

The formation of tryptophane from Witte peptone as discussed by VINES (32) was used to estimate the activity of ereptase. The tryptophane was dissolved in bromine water and treated with amyl alcohol. The activity of catalase was determined by the liberation of oxygen from hydrogen peroxide by the method of APPLEMAN (1).

IV. POTASSIUM DETERMINATION

Potassium was determined by the cobaltinitrite method, the precipitate being measured by titration with potassium permanganate. The micromethod of KRAMER and TISDALL (17) as modified by KERR (15) was employed.

V. SUGARS

Reducing substances were determined by the MUNSON and WALKER method (23). The amount of copper oxide formed in the Fehling's solution was measured by the BERTRAND method (3). The citric acid inversion method described by DAVIS and DAISH (5) was used for determining the total sugars.

VI. MICROCHEMICAL TESTS

The tests used for cellulose were methylene blue and chlorozinc iodide. Orcin and phoroglucin were employed to test for lignin. Calcium pectate was stained by means of methylene blue and ruthenium red. Tests for cutin were made with Sudan III.

Results

I. GROWTH

The growth rate of the plants in all the solutions remained equal until March, by the end of which month a decided gradation in size was apparent. The rate remained equal while the light was deficient, but when the days became longer and sunnier the plants receiving potassium began to grow more rapidly, while the potassium-deficient plants gradually ceased to grow.

The starvation symptoms were small size and dieback, the latter particularly of the lower leaves. Figs. 1 and 2 show their appearance on April 12. Measurements of single plants made on April 14 are recorded in table I The length of the tops was obtained by stretching them out and measuring to the tip of the longest leaf Breadth of

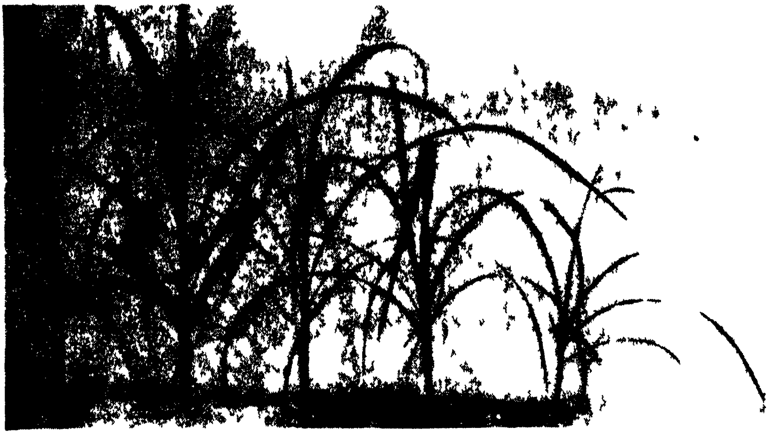


FIG 1 —Appearance of sugar cane plants on April 12 5 5 months old, left to right I, IV, III, II

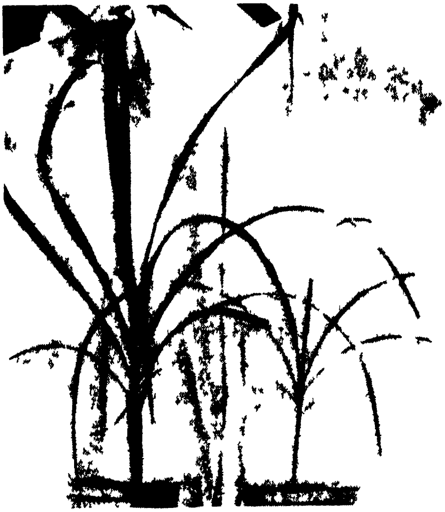


FIG 2 —Sugar cane plants on April 12, left to right I, II

leaf is a measurement taken at the middle of the largest leaf The differences in the weights of the roots are more apparent than real

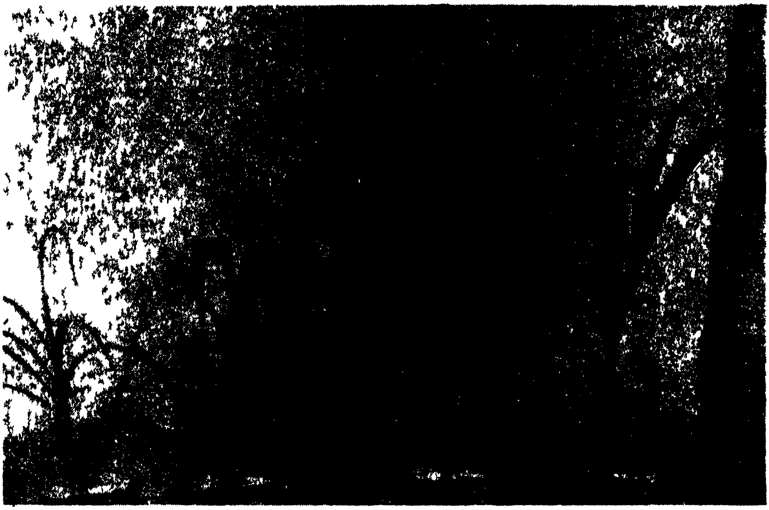


FIG 3 —Sugar cane plants in June, 7 months old, left to right II, III, IV, I

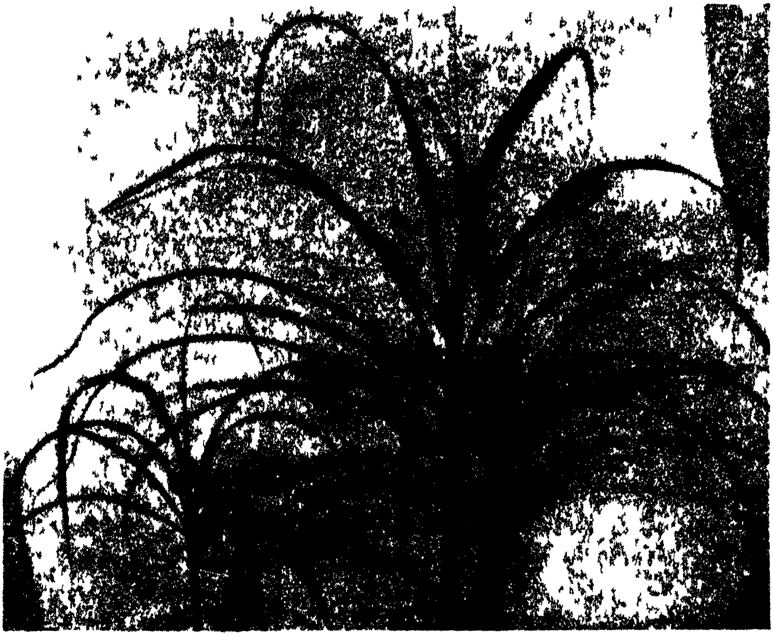


FIG 4 —Sugar cane plants in June, left to right II, IV

because some sand clung to them. This difficulty was obviated in later work by rinsing in 10 per cent sodium chloride.

At the time of collection a great difference in the roots was observed. The roots of plant I were glistening white and had many fine branches forming a dense system. They were tough and strong and lifted completely out of the crock without breaking. Contrasting with this were the roots of plant II, which were discolored, brownish, and were thin with the fibrous roots underdeveloped. These broke easily and it was very difficult to remove them from the crock. Notwithstanding the fact that the tips broke off, the longest roots of II, measured as a mass, were 25 inches in length while those of I were only 20 inches long. The roots of plant III were a little stronger than those of II but showed similar discoloration. Plant IV, however, had roots almost as strong and white as plant I. Although the roots of plant IV measured half as long as plant III, they weighed almost twice as much.

TABLE I

MEASUREMENTS OF CANE PLANTS COLLECTED APRIL 14

PLANT	WEIGHT TOPS (GM.)	WEIGHT ROOTS (GM.)	LENGTH TOPS (IN.)	LENGTH ROOTS (IN.)	LEAF BREADTH (CM.)	STEM GIRTH (CM.)
I	110.5	48.0	67.0	20	3.6	4.9
II	31.5	12.0	55.0	25	2.1	3.0
III	51.0	14.9	59.5	35	2.7	3.4
IV	54.7	26.0	56.5	17	2.6	3.4

FIG. 5.—Roots of plant I as removed from crock on June 2, showing potbound condition.

The second collection was made on June 2, when the plants appeared as in figs. 3 and 4. When the roots were removed it was found that plant I was potbound (fig. 5). Table II shows their fresh weight and table III their size. The roots were washed in 10 per cent sodium chloride and then distilled water. The symptoms of potassium starvation noticed at the time of the first collection were small size and dieback. To these was added another symptom by the time of the second collection, discoloration of the leaves. The upper leaves of plant II were decidedly yellower than the lower leaves. This yellow discoloration was confined to the tissue between the veins, which were green. The other plants were green without discoloration. The incrustation of wax in the region of the nodes was much less in plant II than in the other plants.

TABLE II

WEIGHT OF CANE PLANTS COLLECTED JUNE 2, EXPRESSED IN GRAMS

PLANT	TOPS	BLADES	SHEATHS AND STEMS	ROOTS
I............	187.0	76.0	111.0	118.5
II...........	64.0	40.0	24.0	43.0
III..........	105.5	63.0	42.5	102.0
IV...........	178.0	96.7	81.3	106.8

TABLE III

MEASUREMENTS OF CANE PLANTS COLLECTED JUNE 2

PLANT	LENGTH TOPS (IN.)	LENGTH ROOTS (IN.)	LEAF BREADTH (CM.)	STEM GIRTH (CM.)	NODAL GIRTH (CM.)
I..............	64.5	19.0	4.9	7.2	6.5
II.............	57.2	26.2	2.4	4.5	2.8
III............	63.2	29.2	3.3	5.0	3.9
IV.............	65.2	21.5	4.0	6.1	5.0

The last collection was made July 12, at which time the leaves of plant II were conspicuously yellow while the others were green. The stems showed a gradation in size, as illustrated in fig. 6. This material was used for microchemical tests.

II. ENZYME RESULTS

A. DIASTASE

Tops collected April 14.—As this experiment was performed three times with similar results, an account of a typical set will be given. Lots of powder weighing 0.04 gm. from each plant were extracted in 4 cc. H_2O at room temperature for four hours. To these 1 per cent enzyme solutions were then added 5 cc. of 1 per cent starch solution plus 0.5 cc. toluol and enough sodium phosphate to make them pH 4.4. The 1 per cent solution of soluble starch was prepared according to the directions given by WAKSMAN and DAVISON (33). The cultures were incubated at 42° C. for 24 hours, at which time the results

FIG. 6.—Stems of plants collected July 12, 8.5 months old, left to right I, IV, III, II

shown in table IV were obtained. Since the tube containing material of plant II became red upon the addition of IKI, while that of plant I turned blue-purple, it was concluded that the diastase activity of the potassium-deficient plant was greater than that of the control.

TABLE IV

DIASTASE ACTIVITY AS SHOWN BY COLOR WITH IKI

PLANT	TOPS, APRIL COLLECTION	BLADES, JUNE COLLECTION	STEMS AND SHEATHS, JUNE COLLECTION
I............	Blue-purple	Brown-red	Dark purple
II............	Red	Pink-brown	Yellow
III...........	Purple-red	Pink-brown	Yellow
IV...........	Blue-purple	Red	Red

Plant III was intermediate in activity, while plant IV closely resembled plant I.

Plants collected June 2.—One per cent solutions of enzymes prepared from blades and stems plus sheaths, extracted one-half hour and incubated 24 hours at 40° C., gave the results shown in table IV. The coloration produced by the addition of IKI showed that the potassium-deficient plants had greater diastase activity than the controls, both in the blades and in the stems plus sheaths. The roots of the plants collected June 2, tested in the same way, all gave a dark red color, indicating uniform diastase activity.

B. INVERTASE

0.1 gm. lots of powder plus 5 cc. H_2O plus 5 cc. 3 per cent sucrose plus 0.5 cc. toluol, incubated 24 hours at 41° C., gave the results presented in table V. The amount of invert sugar formed by the invertase of the tops collected in April was about the same in all the plants, showing that the invertase activity of the plants did not vary at that age. By the time of the June collection the activity of the

TABLE V

INVERT SUGAR FORMED BY INVERTASE

PLANT	TOPS, APRIL COLLECTION (MG.)	BLADES, JUNE COLLECTION (MG.)	STEMS AND SHEATHS, JUNE COLLECTION (MG.)
I............	22.1	42.8	35.4
II............	21.6	18.0	39.7
III...........	19.3	22.0	40.1
IV...........	23.2	20.6	31.7

blades of plant I had almost doubled, while that of the other plants remained about the same. The activity of the stems and sheaths had also increased by June, but more uniformly than the blades, since the plants receiving the intermediate amounts of potassium, were the extremes in activity.

C. PEPTASE

Preliminary tests showed pH 8 to be a favorable reaction for cane peptase. This reaction was therefore maintained in the experiment by means of sodium hydroxide with sodium phosphate as buffer.

Cane tops.—0.1 gm. lots of powder plus 5 cc. H_2O plus 0.1 gm. carmine fibrin plus 0.5 cc. toluol at pH 8, incubated at 40° C., began to develop a faint pink color in 3 days. The test was discontinued at the end of one week because the color in all the tubes deepened uniformly, indicating equal activity.

TABLE VI

COLOR RELEASED FROM CARMINE FIBRIN BY PEPTASE IN 5 DAYS

PLANT	BLADES	STEMS AND SHEATHS
I	Deepest pink	Deepest pink
II	Pink	Pink
III	Deeper pink	Pink
IV	Deepest pink	Deeper pink

Cane blades and stems plus sheaths.—These, tested as indicated, began to show a faint pink color in 2 days. The color did not deepen uniformly, but gave in 5 days the gradation shown in table VI, proving that the plants supplied with potassium had greater peptase activity than the potassium-deficient plants.

Cane roots.—These, tested as indicated, liberated color much more rapidly than the tops. In seven hours I and IV were pink but II and III remained light yellow; therefore the peptase activity of cane roots is greater than that of the tops, and both the roots and the tops have greater activity when they have been supplied with potassium.

D. EREPTASE

Preliminary tests showed pH 4.8 favorable for ereptase, and this reaction was therefore maintained in the experiments.

Cane tops.—0.02 gm. lots of powder plus 5 cc. H_2O plus 0.1 gm. Witte peptone plus 0.5 cc. toluol at pH 4.8 were incubated 3 days at 40° C. When tested with bromine water and amyl alcohol the four lots showed equal development of color, hence equal production of tryptophane and equal activity of ereptase.

Cane blades.—These, tested as indicated, all gave equally good tryptophane reactions, indicating equal activity of ereptase.

Cane stems plus sheaths.—These gave strong tryptophane reactions in plants II, III, and IV but only slight in I.

TABLE VII

OXYGEN LIBERATED BY CATALASE IN 0.1 GM. MATERIAL

PART	MINUTES	I (cc.)	II (cc.)	III (cc.)	IV (cc.)
Tops, April 14	1	2.27	0.66	0.82	0.27
	2	4.31	0.91	0.91	0.45
	5	6.81	1.18	1.18	0.75
	10	8.76	1.18	1.18	0.96
Blades, June 2	1	15.06	3.34	6.40	10.10
	2	21.38	4.15	8.21	12.90
	5	30.40	5.05	10.19	16.93
	10	39.24	5.68	11.82	21.02
Stems and sheaths, June 2	1	29.46	9.97	6.05	21.54
	2	42.45	13.17	8.54	28.93
	5	62.66	17.18	10.95	38.09
	10	77.70	20.56	12.99	49.31
Roots, April 14	1	2.36	2.45	2.27	1.73
	2	3.27	3.00	2.55	2.73
	5	4.82	3.64	2.91	4.36
	10	6.00	4.00	3.73	5.73

E. CATALASE

The amount of oxygen liberated from 10 cc. of 3 per cent hydrogen peroxide by 0.1 gm. material was measured in 1, 2, 5, and 10-minute intervals, in duplicate. The results were averaged and calculated to standard temperature and pressure and are given in table VII. This shows that the control plants had the greatest catalase activity, and that in general the gradation in activity was correlated with the gradation in the amount of potassium supplied. A notable exception was found in the stems and sheaths, in which the catalase of plant II was decidedly more active than that of plant III.

III. EFFECT OF EQUALIZING POTASSIUM ON INVERTASE OF CANE BLADES

As shown in table V, the blades of plant I had an invertase activity about double that of the other plants. The question arose as to whether potassium is important for the formation of invertase in cane blades, or merely for its activity. To decide this point, potassium determinations in plants I and II were made, and then enough potassium phosphate was added to plant I to equalize the potassium in both materials. Potassium determinations were made in duplicate, and it was found that the dry weight percentage of potassium of plant I was 3.616 while that of plant II was only 0.497. This necessitated the addition of 1.04 cc. M/10 KH_2PO_4 to 0.1 gm. blade material of plant II and an equivalent amount of sodium phosphate to plant I (to regulate the pH and the osmotic concentration). Blanks were run on both the phosphates. The results, which are given in table VIII, show that even after equalization of the potassium, the activity of plant I remained somewhat more than twice that of plant II, indicating that potassium must play a rôle in the actual formation of invertase.

TABLE VIII

EFFECT OF EQUALIZING POTASSIUM IN CANE BLADES I AND II UPON INVERTASE ACTIVITY THEREOF

LOT	$KMnO_4$ DETERMINED (CC.)	N/10 $KMnO_4$ CALCULATED (CC.)	INVERT SUGAR (MG.)
Blank Na....	1.10	....	....
Blank K.....	1.05	....	....
I............	40.50	39.211	63.6
II...........	18.85	17.714	28.2

IV. MOISTURE DETERMINATIONS

The results of the moisture determinations are presented in table IX, which shows that the amount of moisture in the sugar cane plants varied directly with the amount of potassium supplied.

V. SUGARS

The results of the sugar determinations are presented in table X. In every case plant II had the greatest percentage of sugars, and in general there is a negative correlation between the percentage of sugar and the amount of potassium supplied. The total amounts of sugar do not show a gradation because of the differences in the size of the plants.

TABLE IX

MOISTURE PERCENTAGES IN CANE

PLANT	TOPS, APRIL	BLADES, JUNE	STEMS AND SHEATHS, JUNE
I.............	82.4	73.2	83.2
II............	77.7	70.3	77.8
III...........	79.3	69.5	79.5
IV...........	79.8	74.2	81.0

TABLE X

RESULTS OF SUGAR DETERMINATIONS

PLANT	REDUCING SUBSTANCES			SUCROSE			TOTAL SUGARS		
	Tops	Blades	Stems	Tops	Blades	Stems	Tops	Blades	Stems
	Percentages of sugar in sugar cane								
I	0.200	0.437	0.506	1.923	1.835	2.799	2 123	2.272	3.305
II......	1.230	1.618	1.728	3.130	3.872	3 319	4.360	5 490	5.047
III.....	1.170	1.605	1.753	2.240	3.019	2.766	3.410	4.624	4 519
IV.....	0.858	1.299	1.074	2.276	1.924	2.987	3.134	3.223	4.061
	Total amounts of sugars in entire plants, expressed in gm.								
I	0.2210	0.3321	0.5617	2.1249	1.3946	3.1069	2.3459	1.7267	3 6686
II......	0.3874	0.6472	0.4147	0 9859	1.5488	0.7966	1.3733	2.1960	1 2113
III.....	0.5967	1.0111	0.7450	1.1424	1.9020	1.1755	1.7391	2.9131	1 9205
IV.....	0.4693	1.2561	0.8732	1.2249	1.8605	2.4284	1.6942	3.1166	3.3016

In connection with the extraction for the sugar analyses, observations on the chlorophyll content were made. The alcoholic extracts of plants I and IV were a vivid pure green, while those of II and III were a yellow-olive green.

VI. MICROCHEMICAL INVESTIGATIONS

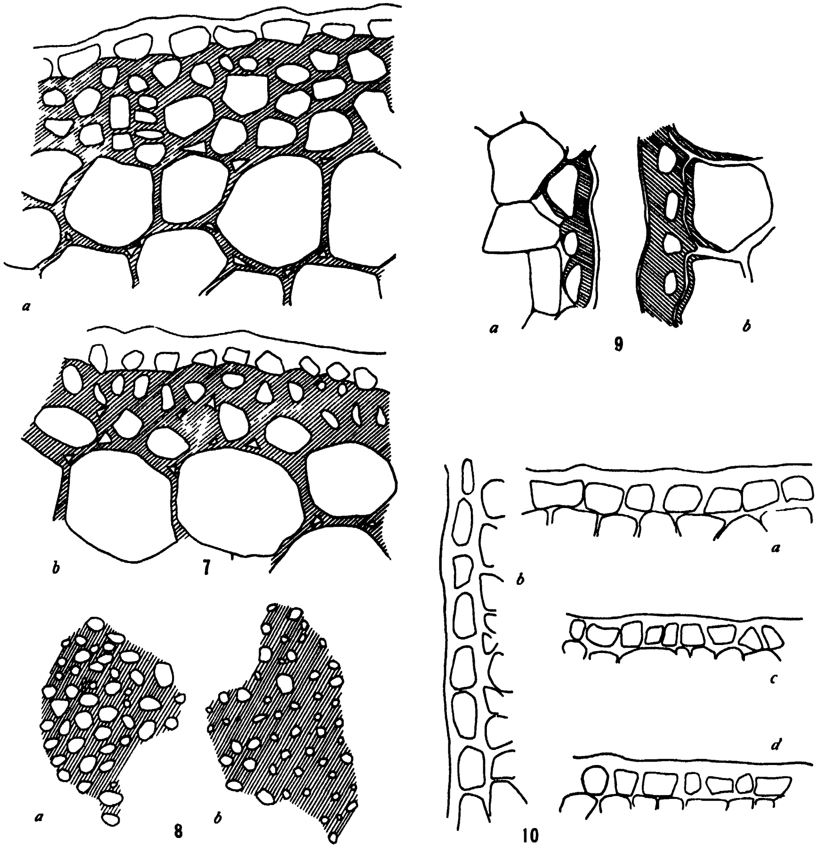
A. LEAVES

Tests on cellulose, lignin, calcium pectate, and cutin were made on cross-sections of equal-aged blades, the sections being made at about the middle of the leaf. There was no evidence that the amount of potassium affected the development of cellulose or calcium pectate, but an increase in cutin and a decrease in lignin were correlated with an increase in the amount of potassium in the nutrient solutions. The subepidermal plates composed of sclerenchyma cells, found just beneath the upper epidermis near the region of the midrib, and the ordinary girders of sclerenchyma found on the lower side of the leaf showed decided differences in the amount of lignin. Figs. 7 *a*, *b* and 8 *a*, *b* show that the sclerenchyma cells in the regions mentioned in plant I had larger lumina, and in general thinner walls than those in the potassium-deficient plants; but the differences in coloring, which cannot be depicted, were much more pronounced. Lignified walls of plants II and III stained a bright red while those of plant I were slightly orange-colored. Cutinized walls of plant I stained deeper red with Sudan III than did the walls of the other plants, which became yellowish red.

Sections cut through the sheath 1 inch from the blade showed that the potassium-deficient plants developed more lignin than those supplied with potassium (fig. 9 *a*, *b*).

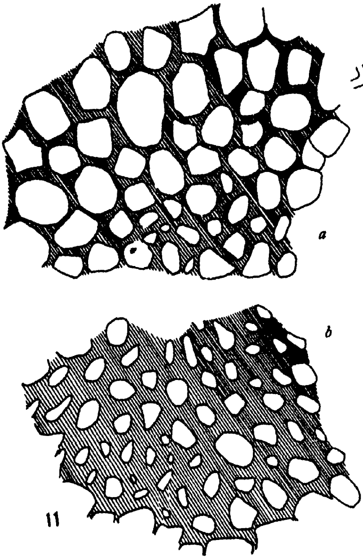
B. STEMS

Stems sectioned at the fourth internode differed in the amount of cutin and lignin and in the size of the cells. The cuticles of plants II and III were thinner than those of plants I and IV (fig. 10 *a*, *d*). The sclerenchyma walls were thinner and paler, and the lumina were larger in the bundle sheaths of plant I than plant II, when taken in the internode (fig. 11 *a*, *b*). The walls of vessels in similar locations were thinner in plants I and IV than in II and III (fig. 12 *a*–*d*). These results are not in accord with those of PURVIS (26), who found the thickness of the xylem walls of *Dactylis glomerata* to be the same with and without potassium. As the diagrams in fig. 12 indicated variation in the size of the cells, several outlines of the vessels near the center of the stems were made, which showed that the vessels of plant II were decidedly smaller than the others, while those of plant



FIGS. 7–10.—Fig. 7, effect of potassium on lignin; cross-sections of blades through upper epidermis showing subepidermal plates of sclerenchyma: *a*, plant I; *b*, plant II; ×35.5. Fig. 8, effect of potassium on lignin; girders of sclerenchyma under lower epidermis of blades, seen in cross-section: *a*, plant I; *b*, plant III; ×35.5. Fig. 9, effect of potassium on lignin; cross-sections of sheaths through lower epidermis: *a*, plant I; *b*, plant II; ×35.5. Fig. 10, effect of potassium on cutin; cross-sections of stems through fourth internode: *a*, plant I; *b*, plant II; *c*, plant III; *d*, plant IV; ×35.5.

III were only slightly smaller. A comparison of the largest parenchyma cells in the fourth internode showed a slight gradation in size. Besides these differences, certain other observations were made: the plants receiving potassium had more wax in the wax bands; there was greater development of anthocyan in the potassium-deficient plants;



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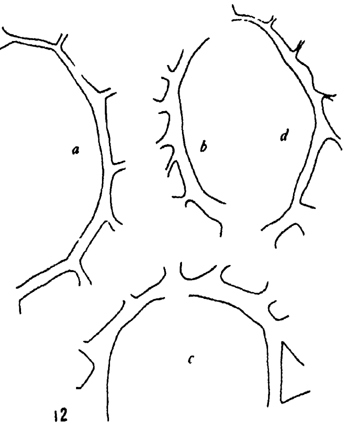

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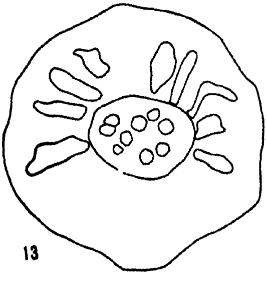

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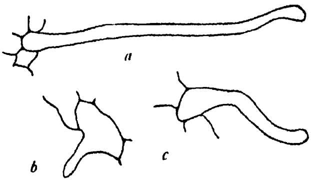

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FIGS. 11–14.—Fig. 11, effect of potassium on lignin; cross-sections of stems through fourth internode, near center, showing portions of bundle sheaths next to phloem: *a*, plant I; *b*, plant II; ×35.5. Fig. 12, lignified walls of vessels near center of stems, cross-sections through fourth internode: *a*, plant I; *b*, plant II; *c*, plant III; *d*, plant IV; ×35.5. Fig. 13, cross-section of root of plant II, 1 inch from tip, showing cavities in cortex and irregular distribution of vessels in pith; ×6.0. Fig. 14, root hairs seen in cross-sections of roots 1 inch from tip: *a*, plant I; *b*, *c*, plant III; ×25.8.

and brown discolorations appeared in the bundles and the parenchyma of plant II.

c. ROOTS

Three striking abnormalities were found in plant II: the almost total lack of root hairs; the irregular distribution of the vessels in the pith (fig. 13); and the presence of large cavities in the cortex (fig. 13). The solid cortex of plant I contrasted sharply with that of plant II, which was full of cavities, possibly similar to the loose tissue noted by SOMMER and SOROKIN (31) in longitudinal sections of the root tips of *Pisum sativum* grown without potassium and with boron. The difference in the size of the hairs on plant I and III is depicted in fig. 14 *a–c*. The endodermis and the sclerenchyma of the stele of plant II stained more heavily than that of plant I, indicating a greater development of lignin, but no differences in the thickness of the walls were noticeable in the sections.

Discussion

The purpose of these experiments was not merely to grow plants with and without potassium and note differences, but to attempt to obtain a graded series correlated with the amounts of potassium in the nutrient solutions. The solutions contained the following amounts of potassium:

SOLUTION	PER CENT	P.P.M.
I	0.07038	703.8
II	0.00000	000.0
III	0.00039	3.9
IV	0.00390	39.0

An examination of the photographs and a study of these data show that positive gradations have been obtained in weight and certain other measurements of size, moisture percentage, invertase activity of blades, peptase activity of blades, stems plus sheaths, and roots, catalase activity, cutin and wax formation, size of cells, and production of root hairs. The size measurements which varied directly with the amount of potassium were weight of tops, blades, sheaths plus stems, roots, length of tops, leaf breadth, and stem girth. Plant IV surpassed plant I in several respects, for example, length of tops on June 2. This, although possibly due to the fact that plant I was pot-bound, indicates that the amount of potassium in solution IV (39 p.p.m.) was almost enough for usual growth. It seems, therefore, that under these cultural conditions sugar cane plants require much less potassium for their usual physiological processes than the amount contained in SHIVE's best solution. This amount is much greater than that determined by other investigators as the minimum potassium requirement for the maximum growth of plants. JOHNSTON and HOAGLAND (14) found that a solution originally containing 4.9 p.p.m. potassium flowing at the rate of 8 cc. per minute per plant was as good for tomato plants as their control, 35.1 p.p.m. BARTHOLOMEW and JANSSEN (2) reported that about 2 p.p.m. is essential for the best growth of oats, cowpeas, soy beans, and cotton; while their maximum growth of sudan grass occurred at 3 p.p.m. PARKER and PIERRE (24) found that the minimum concentration of potassium for the maximum growth of corn and soy beans was 2 p.p.m., or possibly less. Since the results of the present investigation show that 3.9 p.p.m. potassium is decidedly insufficient for the best growth of sugar cane, one may conclude that cane requires more potassium than the other plants mentioned. This is in agreement with the statement made by RUSSELL (29) that sugar-producing plants stand most in need of potassium salts. One possible explanation of the greater need for potassium in sugar-producing plants is found in the evidence presented in this paper that potassium plays a rôle in the formation of invertase in sugar cane.

The plants, of course, all started with a reserve of potassium in the 2 inches of seed piece transplanted with them. However potassium functions in the plant, it seems reasonable to suppose that this reserve would last for a considerable time. As already stated, their growth rate remained equal until March. Four months seems a long time for their potassium reserve to be sufficient for their needs, the truth of which was evidenced by the ability of plant II to equal the others in size and appearance. This might be explained in two ways. Perhaps potassium is important as a catalyst, and as such is not rapidly used up but may act again and again; or perhaps another factor limited the growth of all the plants during those four months. Both possibilities might be true. Because gradation in growth and size was apparent after the days became longer and sunnier, it seems likely that light was the limiting factor. The results obtained in growing buckwheat and soy beans during the fall and spring indicated the same possibility. Perhaps during the fall and winter all the plants had sufficient potassium for their growth processes, which went on slowly because of lack of light. In the spring light ceased to be the limiting factor, however, and the growth rate therefore increased in proportion to the amount of potassium supplied. These results agree with those of JOHNSTON and HOAGLAND (14), who found in their work with tomato plants that shade and low potassium are apparently two limiting factors which operate at the same time; and that the maximum yield would be obtained only by increasing both factors.

The potassium-deficient plants had longer roots than the others (tables I and III). Somehow there was a lack of differentiation and formation of secondary roots, so that evidently all the available food went into the lengthening of the main roots. Other workers have obtained similar results. MICHEELS (20) found that wheat plants supplied with sodium have a greater root length than those furnished with potassium. WALLACE and HUTCHINSON (34) found that the root systems of willow cuttings grown in a solution lacking potassium were more slender and had fewer secondary roots than those supplied with potassium. There are several possible explanations of these differences in root development. The greater percentage of sugars in the potassium-deficient plants may have resulted in the lengthening of the main roots, just as the tomato plants studied by REID (28) had a more vigorous development of roots when the carbohydrate reserve was high. The increase in the amount of calcium and phosphorus with potassium deficiency, as noted later in the work of JOHNSTON and HOAGLAND (14), may be another explanation, since these elements increase root length. REED (27) found that potassium is essential for mitosis; perhaps the cells of the pericycle did not have enough potassium for division to occur and therefore few secondary roots started. Or it may be, as suggested by MOLISCH (21), that potassium is important in building up the plasma of the growing points; there may not have been enough potassium in the plants for the development of many secondary growing points. This possible relationship of potassium to the formation of protoplasm in the growing points may be correlated with the activity of peptase, as discussed later. Probably not one but all of these factors contributed to the greater length of the main roots and the lack of differentiation of branch roots in the potassium-deficient plants. What are the physico-chemical processes which result in the beginning of branch roots? Whatever they are, lack of potassium seems to upset the physiological balance, as few were seen by microscopic observation. The development of cavities in the roots of plant II, as seen in cross-section (fig. 13), may be due to the death of the cells or possibly to differences in the cell wall constituents. The occasional dropping out of entire rows of cells was noted by SOMMER and SOROKIN (31), in longitudinal sections of the root tips of *Pisum sativum* grown without potassium and with boron.

Table XI shows the enzyme results obtained. It should not be surprising that all enzymes are not affected in the same way. Life processes in a plant are so interwoven and concatenated that an upset of one reaction in one direction may result in derangements of many other reactions, and it may very well be that cause and effect can never be disentangled.

TABLE XI

SUMMARY OF ENZYME RESULTS

ORGAN	DIASTASE	INVERTASE	PEPTASE	EREPTASE	CATALASE
Tops	—*	o*	o	o	+*
Stems and sheaths	—	o	+	o	+
Blades	—	+	+	o	+
Roots	o	....	+	....	o

* + =potassium increases action; — =potassium decreases action; o =potassium seems to have no effect; blank =not tested.

The diastase results presented in this paper are similar to those obtained by DOBY and HIBBARD (7) with the leaves of the sugar beet. They are also similar to the present writer's unpublished results with wheat and soy beans, and with buckwheat grown in the spring. Buckwheat grown in the fall and therefore with deficient light showed better diastase activity with the plants receiving potassium than with the potassium-deficient plants. The suggestion proposed by DOBY and HIBBARD that the greater diastase activity in the plants grown with insufficient potassium might be due to starvation, seemed not to explain the phenomenon fully. Since diastase is considered to be active in starch formation as well as digestion, and since higher percentages of sugars were obtained in the potassium-deficient plants, is it not possible that the increased diastase activity of the plants starved for potassium is explainable by Knudson's theory of the quantitative regulation of enzymes? Knudson (16), working with *Aspergillus niger* and *Penicillium* spp. grown on synthetic media, found that they did not form tannase, but after the addition of tannic or gallic acid tannase was formed. Since a greater amount of tannase was produced when there was a larger amount of tannic acid in the medium, it was suggested that the production of enzymes is regulated quantitatively by the amount of the substrate present. Other examples of the effect of the composition of the medium upon the development of enzymes are reviewed by Waksman and Davison (33). In the present case the greater diastase activity of the potassium-deficient plants may have been caused by their increased percentage of sugars, which constitute the substrate necessary for the formation of starch by diastase.

TABLE XII

Growth from April to June (estimated from different plants)

Plant	Leaf breadth (cm.)	Stem girth (cm)
I..........	1.3	2.3
II	0.3	1.5
III	0.6	1.6
IV.........	1.4	2.2

Possibly sugar cane resembles the tomato plants studied by Johnston and Hoagland (14) in absorbing more phosphorus when the supply of potassium is deficient. If this is true for cane, possibly the increased amount of phosphorus is another factor in causing the greater diastase activity in the potassium-deficient plants, since phosphorus is known to favor that enzyme.

Diastase activity may be correlated with the growth rate. Subtracting the results in table I from those in table III, the data presented in table XII are obtained. The growth of the stems as shown by their girth from April to June decreased in this order: I, IV, III, II. Table IV shows the diastase activity of stems collected in June decreased in this order: II–III, IV, I. Table X shows the percentage of total sugars in stems and sheaths decreased in the same order. Breadth of leaf decreased in the order: IV, I, III, II. Table IV shows the diastase activity of blades collected in June decreased as follows: II–III, I, IV. The percentage of total sugars in the blades decreased in the order: II, III, IV, I. In general, the more rapidly the plant grew, the less the diastase activity. Possibly the increased growth of the control plants used up the sugars in the formation of protoplasm, thus leaving no surplus sugars to increase the diastase activity by quantitative regulation; but lack of potassium in plants II and III curtailed their growth, which resulted in the accumulation of an excess of sugars, which in turn caused their greater diastase activity.

The weak diastase activity of all the roots was not surprising since the root does not manufacture starch. This, as well as the results with invertase, illustrates the fact that different organs of the same plant react differently.

The same organs react differently at different ages. Tops collected April 14 showed no effect of potassium upon invertase, but by June 2 the blades of plant I had developed a much greater activity while that of the other plants remained about the same. The activity of the stems plus sheaths also increased, but more uniformly than the blades, as the intermediate plants were the extremes in activity. Peptase is another illustration of the effect of age. Tops collected in April showed equal activity while all organs collected in June showed increased activity with increased potassium. External symptoms are undoubtedly the outward expression of internal derangements, some of which are at first too delicate for determination by our clumsy methods, but with increased age the unbalanced physiological conditions become more pronounced and hence are more readily determined.

The invertase results in the blades led to an attempt to equalize the potassium in the material, as explained in section III of the results. The blade materials contained different amounts of potassium which might be directly responsible for the results. It was thought that if equalizing the potassium resulted in equalizing the invertase activity, then potassium would be considered important as a coenzyme or regulator of invertase activity. But if equalizing the potassium did not result in equal invertase activity, it would be concluded that potassium is somehow necessary in the actual formation of invertase. The results presented in table VIII certainly support the second theory, since the activity of plant I after the equalization of potassium remained somewhat more than twice that of plant II.

If the invertase of blades differs, why does not that of the stems? Why are the results in all the stems more like the results in the blades of plant I than plant II? Is it possible that the stems contain more potassium than the blades? Unfortunately these determinations could not be made.

Table VI shows that the activity of peptase was greater in the plants receiving potassium than in the potassium-deficient plants. This was even more noticeable in the roots than in the parts above ground. The deficient peptase activity would naturally cause a decrease in the formation of proteins and hence in the development of protoplasm. This seems an adequate explanation of the lack of development of secondary roots, the cessation of growth of the tops, the dieback of the leaves, and possibly even of the derangements in the formation of chlorophyll.

The importance of the catalase results depends upon the importance attached to catalase as an enzyme. MORGULIS (22) has claimed that it is not a measure of metabolic activity. However that may be, catalase activity was found to vary directly with the potassium supplied and the growth responses, except in the roots.

It has been shown previously that the enzyme response varies with the age of the plant and the organ studied. Other experiments not here reported have shown that it varies with different species of plants, studied in similar ways. The peptase activity of the blades of soy beans varied directly with the amount of potassium; there was no difference in the leaves of buckwheat at the age studied. Buckwheat leaves had greater activity of ereptase with increasing amounts of potassium, but soy bean blades showed decreased activity with greater potassium content. The catalase activity of buckwheat varied directly, that of soy bean blades varied inversely, while that of wheat seemed unaffected by the amount of potassium supplied. It is evident that a deficiency in potassium upsets the balance of physiological processes governed by enzymes. It seems more reasonable to expect that the balance in different plants would be upset differently, than that all plants would react in the same way.

Added to the direct effect of potassium deficiency in causing decreased synthesis of proteins, cessation of growth, etc., there may be an indirect effect in the influence of the lack of potassium upon the intake of other ions. JOHNSTON and HOAGLAND (14) found that tomato plants grown with low potassium content in the nutrient solution absorbed greater amounts of calcium, magnesium, and phosphorus than plants supplied with the full amount of potassium. Undoubtedly the increase in these three ions influences the physicochemistry of the potassium-deficient plants, if this increase is characteristic of all kinds of plants grown with a small amount of potassium. Possibly the greater length of the roots of the plants starved for potassium was influenced by the increased amounts of calcium and phosphorus, since these elements are known to increase the length of roots. The phosphate ion is known to be favorable to diastase activity, since the normal transformations of starch are somewhat dependent upon phosphorus. This may be one factor in influencing the greater diastase activity of the potassium-deficient plants. An increase in the magnesium content might be detrimental since magnesium is known to be toxic at times. The influence of all the essential elements must therefore be considered when studying the effect of the absence of one mineral nutrient. It may be that the problem of the rôle of potassium will be solved only after the equalization of all the other ions within the tissues of the plant.

The larger moisture percentage of the plants supplied with potassium (table IX) is perhaps due to their larger root system, or possibly to differences in colloidal water-holding capacity. It may also have resulted from their growth rates, since increased water content usually accompanies more rapid growth. Whatever the cause, it certainly is correlated with the amount of lignin and cutin. Deficient water supply in a cell would produce conditions favorable for condensation and hence elaboration of lignin; or the lower moisture content of the samples of the potassium-deficient plants may merely have resulted from the presence of more lignin in the samples. Transpiration from plants II and III may have been greater than from I and IV because of the decreased cutin in the former. The influence of potassium in governing the turgor of plants has been mentioned by COPELAND (4) and by WEEVERS (35).

The decreased water content of the potassium-deficient plants may help to explain why plants starved for potassium sometimes show less resistance to frost than do plants supplied with potassium. The statement that potassium protects plants against frost has been made by LOEW (19), by PATTERSON (25), and by other workers, although the exact way in which it acts in this respect has not been definitely determined. The winter killing of plants is generally considered to be due to drying as well as to freezing, and it stands to reason that, other things being equal, plants with a lower water content would dry out more rapidly, especially when their cuticle is not well developed. Another factor involved in the resistance of plants to low temperatures is the nature of their proteins. HARVEY (10) found that certain changes in proteins occur, which are partly hydrolytic and which result in making them less easily coagulated. Perhaps peptase is one of the enzymes needed to catalyze these changes, which would therefore take place more slowly in the potassium-deficient plants. In short, potassium may increase resistance to cold by giving plants a greater water-holding capacity, a better developed cuticle which resists drying out, and more active peptase which is important in catalyzing certain changes in proteins in the process of the hardening of plants.

The sugar analyses showed that the potassium-deficient plants had a greater percentage of total sugars and reducing sugars than the plants with a larger amount of potassium. Table X shows a regular gradation; the less the potassium, the more the sugar. Sucrose gave a similar gradation except in the stems (table X), where, however, plant II had the largest amount. It is interesting that in the stems neither the sucrose content nor the invertase activity varied greatly, while in the blades the largest sucrose content was found in the plants having the least invertase activity. Does the theory of the quantitative regulation of enzymes fail here; or was the potassium content of plant II insufficient to produce much invertase? Perhaps the abundance of hexose may be responsible for the large amount of sucrose, because a great quantity of the substrate may result in the formation of a large amount of the end product even though the enzyme is not very active.

Why do the potassium-deficient plants contain more sugar than the controls? It might presumably be due to the decreased anabolic or increased catabolic activity of the enzymes, which build sugars into higher compounds, or the increased anabolic activity in the enzymes of photosynthesis, or a combination of these. It will be remembered that peptase showed greater activity with increased potassium content. Perhaps the decreased protein synthesis due to the deficient activity of the enzyme peptase may explain the piling up of the sugars in the potassium-deficient plants. It would also explain their decreased growth. This piling up of carbohydrates with poor nutrition has been found by other workers. KRAYBILL stated in an address to the Botany Club of the University of Chicago (unpublished) that he has found increased carbohydrates with decreased nitrogen, sulphur, and phosphorus. HARTWELL (9) found increased starch with decreased potassium and other factors which inhibit growth. JANSSEN and BARTHOLOMEW (12), working with tomato plants, found that high nitrogen and high sugars were correlated in the blooming stage of the low potassium plants, and thought that this might be due to a lack of condensation of these compounds to more complex forms. There is need, therefore, for more work with enzymes. A quantitative measurement of peptase, with the potassium equalized as in the invertase work, might prove suggestive.

Another possible explanation of the accumulation of sugars in the potassium-deficient plants is that proposed by JOHNSTON and DORE (13) in connection with their studies of boron deficiency. They found that tomato plants starved for boron had a greater sugar content in the leaves than the control plants; and since they found phloem necrosis in the petioles and stems of the boron-deficient plants, they thought that the broken-down conducting tissues might be responsible for the accumulation of carbohydrates. Since phloem contains living protoplasm, it might suffer from any severe starvation. In the present investigation only slight evidence of derangements of the phloem was found in the potassium-deficient sugar cane plants: the occasional reddish discoloration of some of the cells of the phloem in the leaves, and the brown discolorations in the bundles of the stems.

Since the potassium-deficient plants have larger percentages of sugars than the controls, one might hastily conclude that greater sugar production would result from starvation for potassium. Table X shows this to be erroneous. While the percentages vary inversely with the amount of potassium, the total amounts vary directly since the control plants are the largest in size.

The increased sugar content of the potassium-deficient plants may help to explain why they are more susceptible to disease. It may also cause their greater development of anthocyan.

Early workers claimed that potassium is essential for photosynthesis. Do these results necessarily disagree? If the percentage of sugars present at a given time may be used as a measure of photosynthesis, then it would certainly seem that the potassium-deficient plants carry on that process better than the plants supplied with potassium. But it has already been shown that decreased peptase activity accompanies deficiency in potassium and may be responsible for the accumulation of sugars. The piling up of sugars might conceivably result in a decrease in photosynthesis, since the accumulation of end products is known to decrease the rate of catalytic reactions. This is not essential to explain the decreased growth, since the deficiency in the protein enzymes could account for that. The results reported in this paper, therefore, neither prove nor disprove the theory that potassium is essential for photosynthesis. That increased sunlight hastened the growth of the plants supplied with potassium, but not those starved for potassium, might be indirect evidence in favor of the theory. More facts regarding the chemical nature of the photosynthetic process are needed before the effect of potassium can be studied directly. The results certainly support the theory that potassium is needed in the synthesis of proteins.

In general, the effect of the lack of potassium seems to be noncoordination, since the action of some enzymes is intensified and of others lessened; but one must not conclude that the function of potassium is the coordination of enzymes without first studying the effects of other essential elements upon enzymes. Lack of potassium stops growth, causes dieback, and decreases chlorophyll formation.

It may be that the cessation of growth is caused by a decrease in the manufacture of proteins, since deficiency in potassium has been shown to interfere with the activity of peptase. Perhaps the decreased formation of proteins results in the accumulation of sugars, which in turn causes increased diastase activity due to the quantitative regulation of enzymes. This increased diastase activity may indicate a possible increase in the activity of certain other enzymes which lead to the formation of lignin. Greater amount of lignin, poorly developed root system, differences in colloidal water-holding capacity, and other factors would lead to a decreased moisture content. This insufficient supply of water together with the deficient protein formation may explain the dieback of the leaves. Possibly the decreased activity of peptase is important also in explaining the underdevelopment of the secondary roots, and it may even explain the derangement in the formation of chlorophyll. If these derangements of the physiological processes in sugar cane, which have been indicated in the data here discussed, may be thus linked together in a chain of cause and effect, then they present a plausible explanation of the symptoms of potassium starvation.

Summary and conclusions

1. This paper deals with the effect of varying amounts of potassium upon the growth, enzyme activity, moisture percentage, sugar content, cellular structure, and microchemistry of sugar cane.

2. Sugar cane started from cuttings in October and transplanted in November began to show symptoms of potassium starvation in March.

3. The symptoms of potassium starvation obtained were decreased growth, dieback, and deficient development of chlorophyll.

4. It is possible to secure a gradation in growth correlated with the gradation in the amount of potassium supplied.

5. The size measurements which varied directly with the amount of potassium were: weight of tops, blades, sheaths plus stems, roots, length of tops, leaf breadth, and stem girth. The potassium-deficient plants had longer roots than those supplied with potassium.

6. The amount of potassium needed by sugar cane for its usual

physiological processes is less than that contained in SHIVE's best solution.

7. An amount of 3.9 p.p.m. was found insufficient for the best growth of sugar cane.

8. Quantitative determinations were made of the activity of diastase, invertase, peptase, ereptase, and catalase of tops, blades, sheaths plus stems, and roots.

9. Potassium may increase or decrease enzyme activity or have no effect thereon.

10. The effect of potassium upon enzyme activity varies with the kind, age, and the organ of the plant studied.

11. The potassium-deficient plants had greatest diastase activity in tops, sheaths plus stems, and blades. The activity in the roots was equal in all the plants.

12. The invertase activity in the blades was greater in the control than in the potassium-deficient plants. The activity of the stems plus sheaths was equal.

13. The peptase activity of the blades, sheaths plus stems, and roots was greatest in the controls.

14. The ereptase activity was the same in all the plants.

15. The catalase activity was greater in the plants supplied with potassium, with the exception of the roots, in which the activity was equal.

16. Since equalizing the potassium content of cane blades does not equalize the invertase activity thereof, it seems likely that potassium may play a rôle in the formation of that enzyme.

17. The tops of the potassium-deficient plants had a smaller moisture content than the controls.

18. The potassium-deficient plants had greater percentages of total sugars, reducing sugars, and sucrose than the plants supplied with potassium.

19. Greater lignification occurred in the potassium-deficient plants, while greater cutinization was found in the plants supplied with potassium.

20. Abnormal distribution of vessels in the pith of the roots, small size of the vessels and the parenchyma cells of the stems, large

cavities in the cortex of the roots, and underdevelopment of the root hairs were found to attend the lack of potassium.

21. Suggestions are made regarding the causes of the symptoms of potassium starvation, as well as the greater resistance toward low temperatures and diseases found among plants supplied with potassium.

22. These derangements in the morphology and the physiology of sugar cane must be concatenated and an attempt is made to link them together in a chain of cause and effect.

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STAMINATE FLOWER OF ECHINOCYSTIS LOBATA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 396

WARD L. MILLER

(WITH PLATES XIII–XVI)

Introduction

For a long time the interest of botanists and also their ability to interpret have been challenged by at least three morphological situations in the Cucurbitaceae: (1) the epigynous character and the method by which the pistillate flower develops it; (2) the stamen situation which involves some stamens apparently complete and others apparently only half complete; and (3) the taxonomic position of the family which must be determined by the balance, or lack of it, between family characters peculiar to the Sympetalae and those peculiar to the Archichlamydeae. One of these situations, the second, has for nearly a century been a subject of controversy that has been intense and at times almost bitter; and that the matter is still in dispute is evidenced by the recent work and conclusions of HEIMLICH (6) on the subject.

The present investigation of the staminate flower of *Echinocystis lobata* has revealed a number of morphological details which are significant in that they contribute to the interpretation of all three of these situations; to the first indirectly, but to the others directly. An account of these details and of their significance is therefore timely.

Controversy over the interpretation of stamens in the Cucurbitaceae must have begun soon after the first taxonomic description of the family appeared in print, for even the more important formal papers on the subject date back as far as 1855, when NAUDIN (18, 19) expressed his conviction that the large tetrasporangiate stamen was normal, while the smaller bisporangiate one had resulted either from the abortion of the missing half or else from the splitting of a large stamen into two. Two years later PAYER (20) presented the opposing view, that the small bisporangiate stamen was normal, while the large tetrasporangiate stamen had resulted from the union of two normal ones. Not until 1875 did the subject come prominently before the botanical public again, when both EICHLER (4) and VAN TIEGHEM (24) published their accounts of the stamen in this family. Both investigators looked upon the tetrasporangiate organ as being double (VAN TIEGHEM uses the expression "cinq termes doubles" as composing the typical androecium), although they disagreed over the method by which doubling might have occurred. Three years later BAILLON'S paper (1), which had been read before the French Association, was published. For NAUDIN and his supporters this paper contained an emphatic criticism, followed by the author's own interpretation of cucurbitaceous stamens, the latter based for the most part on his investigation of *Bryonia*, and essentially in accord with the interpretation of PAYER. BAILLON referred to the work of DUCHARTRE and to that of DECAISNE, both of whom agreed with NAUDIN, although DECAISNE was obviously ambiguous in many of his statements; he referred as well to TISON whose work on *Thladiantha* bore out his own conclusions. Again in 1886 BAILLON'S position on the stamen situation was published (2). MOREAU (17) reported briefly on an investigation of numerical variations among floral parts of *Bryonia* in 1914. The most recent morphological contribution has been made by HEIMLICH (6), who describes the androecium of *Cucumis sativus* as consisting of two complete stamens and one half-stamen, his interpretation therefore reaffirming the older one of NAUDIN.

Most of the recent work with the Cucurbitaceae has been done, not on floral development, but on cytological aspects of sporogenesis, pollen tube development, and embryology. CASTETTER (3), HEIMLICH (7), KIRKWOOD (10), and KOZHUKHOV (11) have investigated microsporogenesis; while KRATZER (12), LONGO (14), and Miss TILLMAN (23) have worked on various features of the embryo sac and on development of the seed. The pollen tube has received attention from KIRKWOOD (9), LLOYD (13), and LONGO (15). Both KIRKWOOD (8) and LONGO (16) have made embryological studies, and HAGEDOORN (5) has contributed a paper on parthenogenesis. And to these morphological contributions might well be added the two papers of TIEDJENS (21, 22) on sex ratios and on the effects of environment on fruits.

Investigation

MATERIALS

Staminate inflorescences of *Echinocystis lobata* were collected in the summer of 1927 at Grove City, Pennsylvania, and were fixed in formalin-acetic-alcohol. Young inflorescences were fixed without dissection at all, but from the older ones flowers were isolated and treated individually. The long sepals and petals were cut away from most of the mature flowers, so as to avoid the entrapping of air bubbles and to facilitate fixing, infiltration, and general handling.

Sections 5 μ in thickness were used. Part of these were stained with iron-haematoxylin and part with a safranin light-green combination. Safranin was used in 50 per cent alcohol while light-green was used in 95 per cent alcohol. After sections had stood in safranin they were transferred to tap water, where they were left for 10–15 hours before being run up to the light-green.

MATURE FLOWER

The broad and somewhat flat staminate flower of *Echinocystis lobata* has its receptacle cup, which is commonly called the calyx, extending upward to about two-thirds or three-fourths the length of the short androecium. The receptacle bears only a few scattered hairs on its outer surface and these are mostly non-glandular, but its inner surface is densely covered by short, multicellular, glandular hairs. The sepals are six in number, long, slender, acute, and wide spreading. They are sparsely covered, chiefly at the basal ends, by hairs which are multicellular and non-glandular. The petals, which are also six or rarely seven in number, alternate with the sepals. Unlike the latter, the petals are flat and ribbon-shaped, rounded at the tips and white in color. Although they too are long, they spread less widely than the sepals, and near their juncture with the receptacle cup they come in close contact with one another by their edges, thus forming the short corolla tube. On both their surfaces the petals bear simple multicellular hairs, many of which seem to be glandular, although the terminal cells are but slightly if at all enlarged.

The androecium is a complex structure, almost spherical in shape excepting for its two short basal stalks. Its surface is lobed and strongly contoured by reason of the six S-shaped microsporangia (fig. 1), which ultimately form the three locules. If, as is commonly done, the number of stalks and of locules be taken as indicators of the number of stamens present, then the flower has two stamens, one with two locules (four sporangia) and the other with but one (two sporangia). Both stamens adhere to each other by the inner faces of their connectives, however, so that, above the two short stalks, the whole androecium seems to be a single fleshy organ.

ORIGIN OF FLORAL PARTS

The flower is first recognizable as a hemispherical mass of meristematic tissue (fig. 2). From the very first, however, the outermost cell layer is differentiated enough to be visibly distinct, and it is continuous with the epidermis of that cauline member of the inflorescence which bears the flower. In the inflorescence there are neither leaves nor bracts which subtend individual flowers. Very early the shape of the flower primordium changes from that of a hemisphere to that of an inverted, truncated cone whose truncated peak is to become the flower pedicel, and whose base is to become the receptacle, giving origin to perianth and stamens. The base of the inverted cone, due to an upgrowth of its circular margin, becomes slightly concave (fig. 3) and subsequently cup-shaped, with six marginal, hemispherical swellings. The latter are to develop finally into the six sepals. Almost immediately the petal primordia appear, six (or rarely seven) in number, alternating with the sepal primordia, and projecting from the inner surface of the cup toward the flower center (fig. 4). While the juncture of petals and receptacle is actually at a lower level than that of sepals and receptacle, it is morphologically at a higher level, since it is the inner one of the two levels and therefore nearer the tip of the floral axis.

After the perianth has thus started, the stamens appear. These, like both sepals and petals, are first recognizable as dome-shaped swellings of meristematic tissue on the inner surface of the concave receptacle. They are nearer the floral axis than are the perianth members, however, and from the first are of noticeably greater diameter (figs. 5, 6). Although the mature flower has what seem to be but two stamens, the embryonic flower has three separate stamen primordia (fig. 8). Two of these arise close together on the same side of the flower, while the third is opposite them and about equidistant from both. Growth of these primordia is so rapid that the first relative distances between them are soon altered, and they come later to occupy positions on the receptacle floor almost equidistant from one another. This change occurs so early that it is impossible to determine from position alone what the exact arrangement of stamens is, relative to that of petals or of sepals. And neither can their arrangement be determined, in the staminate flower, from their vascular supply, because here, as will be shown later, there is no constant association between stamen bundles and perianth bundles.

The situation in the pistillate flower is sufficiently different from that in the staminate, however, to constitute a clue which seems to determine the matter of stamen arrangement. The pistillate flower has three staminodia which arise exactly as do the stamens in the staminate flower, that is, two close together and the third opposite them around the circumference of the receptacle base. Since these organs always remain small while the rest of the flower is growing, the danger of their being crowded out of original positions is much reduced compared with that in the other flower. Moreover each staminodium is supplied by a single bundle, which, in the part of the receptacle that has grown up around the ovary, is distinctly associated with the vascular supply of a perianth member. Here then is the kind of evidence that can determine the question of arrangement for the pistillate flower; if this evidence can equally well be applied to the problem in the staminate flower, that problem too can be solved. In the pistillate flower the two staminodia which arise close together can be traced by their vascular supply to two bundles which supply adjoining perianth members of different cycles (petal and sepal). The third staminodium, from the same kind of evidence, lies opposite another petal. This evidence indicates that the three staminodium primordia are members of two different cycles; two, lying opposite the petals, are in an upper cycle, while the third, lying opposite a sepal, is in a lower one.

The presence of staminodia in the pistillate flower and the presence of a pistillodium in the staminate flower of other genera (for example, *Cucumis*), would lead one to anticipate a pistillodium in the staminate flower of *Echinocystis lobata*, but none occurs. There are no visible evidences at all of carpels in the staminate material available for this study.

RECEPTACLE

Activity in the receptacle does not cease with the mere production of the primordia of floral leaves and stamens, for no sooner are these organs well started than the receptacle elongates noticeably, carrying sepals and petals up and over the young stamens (figs. 7, 11, 13, 15). It is of some significance whether this elongation results from activity of a limited zone which retains its original meristematic properties, from relatively unrestricted cell division throughout the entire receptacle, from cell enlargement alone, or from some combination of these alternatives; for, among the host of perigynous and epigynous flowers, there is doubtless considerable variation in this feature which may have phylogenetic, ecological, and (in pistillate flowers) even economic importance. In the case of *Echinocystis* the early elongation of the receptacle, up to the stage represented by fig. 15, is due almost exclusively to cell division in that part of the organ between stamen and petal bases. This is evidenced not only by the fact that receptacle cells of a half-grown flower are more numerous and but slightly larger than those of a young flower, but also by the fact that mitotic figures are numerous in the material (figs. 16, 17, 18). The figures most frequently met are those of transverse divisions (fig. 16) which result in elongation of the receptacle. A few are longitudinal (fig. 17), however, resulting in increased thickness, and a few more are tangential, resulting in increased circumference. Mitotic activity, rather than being restricted to a limited meristem or cambium, is general throughout that part of the receptacle outside the stamen cycles; and up to the time of complete cessation of figures the latter are equally numerous in both upper and lower levels.

The decline and final cessation of cell division in the receptacle, however, does not mark the end of that organ's growth; in fact, the receptacle may be no more than half-grown when mitosis stops. Subsequent to that time elongation is a matter of cell growth only (figs. 18, 19). Elongation in this second phase is no more localized than were the mitoses which preceded it, for cells at both high and low levels contribute to it. When mature the receptacle extends up about three-fourths the length of the stamens, constituting the so-called "calyx cup."

As the receptacle approaches maturity it becomes spongy in texture, due to the development of schizogenous, intercellular spaces (fig. 20). Such spaces are more extensive in the upper part of the organ than they are in the lower, and their gaseous contents are continuous with the external atmosphere through numerous stomata (fig. 21), which are irregularly scattered over the outer surface. No stomata have been seen on the inner surface. Chloroplasts are present in parenchyma cells throughout the receptacle, but as a rule none occurs in the epidermis.

Epidermal hairs are numerous on the receptacle, particularly over its inner surface. Here the hairs are simple, multicellular, and glandular, the glands consisting of one, two, or several cells (figs. 22, 23). Hairs with multicellular glands are confined to the lower third of the receptacle cup, while those with one- or two-celled glands are scattered, without other limitations than that they are more numerous over the upper part. It is characteristic of all glands above the lower extremities of the anthers that they contain an abundance of coarse granules which stain deeply with safranin (fig. 22). No microchemical tests were made to determine the nature of these granules, but it is probable that they represent one or more secretion products in their pre-secretion phases. It is equally characteristic of all glands below the anthers that they lack these conspicuous granules. Their large nuclei and their densely staining cytoplasm, however, strongly indicate their secretory function (fig. 23). On the outer surface of the receptacle hairs are much less numerous than on the inner surface. What few hairs do occur are simple and mostly non-glandular (fig. 24), although now and then one finds a glandular hair similar to those high up on the inner receptacle surface.

Twelve vascular bundles traverse the receptacle cup vertically. No well defined branches come from any of these twelve bundles excepting in the upper part of the receptacle, and even then not all of the twelve branch. Here and there groups of elongated cells diverge out from a bundle for a short distance, but invariably the cells contain protoplasm and are differentiated in almost no other feature than their dimensional proportions and their position. Near the upper limit of the receptacle, just below the level where sepals and petals become distinct, six alternating bundles of the total twelve branch in a very definite fashion, giving rise to bundles that supply the perianth members as will be described later. Here also a very few of the vertical bundles may be connected in twos by slender, oblique vascular strands.

CALYX

All six of the sepals are separate throughout their entire length, and therefore there is no real calyx cup. As in the receptacle so in the sepal, elongation results at first from cell division (figs. 25, 26), and finally from cell growth (fig. 27). There are two important differences, however, between the elongation of the receptacle and that of a sepal. In the first place mitotic figures in a young sepal are prevailingly those of transverse divisions, while longitudinal and tangential divisions are exceedingly rare. As a consequence the sepals grow up into long, slender, widely divergent organs, ranging in their cross-sections from elliptical through triangular to circular. In the second place mitotic activity continues for a much shorter time in a sepal than in the receptacle, so that the former's growth is almost exclusively the result of cell enlargement. Here, as also in the receptacle, both mitosis and cell growth are general in their distribution; there is no restricted meristem.

All cells of the epidermis and of the inclosed chlorenchyma are large, and their cytoplasm, due to the development of large vacuoles, becomes very diffuse. The epidermis is thinly cutinized and is broken on the under surface by scattered stomata. While hairs are borne on both upper and lower surfaces they are few in number, and are mostly confined to the proximal or basal portions of the sepals. In structure they resemble the external hairs of the receptacle (figs. 22, 24).

At the base of a sepal the chlorenchyma exhibits the same spongy structure as does that of the receptacle; but out away from the base the intercellular spaces diminish progressively until, at the distal end of the organ, they are nothing more than mere breaks at the cell corners. All parenchyma cells between epidermis and vascular tissues are chlorophyll-bearing.

A single vascular bundle enters each sepal from the receptacle and traverses its entire length. Infrequently this bundle branches and then the two resulting bundles extend to the sepal tip. Xylem is visibly differentiated to within one cell layer of the epidermis at the sepal tip, but visible differentiation of the phloem ceases somewhat farther back than that.

COROLLA

Both mitosis and cell enlargement are responsible for the growth of corolla tube and corolla lobes, although it is not until near their maturity that cell enlargement becomes a noticeable factor (figs. 28–31). Long after cell division has ceased in the sepals it continues in the corolla. The majority of mitotic figures are those of transverse divisions, but many are longitudinal and many others tangential.

It has been commonly accepted that the corolla lobes of sympetalous flowers have their origin as has been described for *Echinocystis*, and that the corolla tube develops only after meristematic activity is resumed in the receptacle immediately below the lobe bases. Such a resumption of activity would cause the whole region to elongate as a cylinder, bearing the separate lobes on top. While this method of growth may apply to many, or even to most, sympetalous flowers, it hardly applies here. There is of course a corolla tube, but it does not possess the smooth continuity that would result from a continuous ringlike meristem at the juncture of corolla and receptacle. Rather it is a tube composed of individual vertical units, clearly marked off from one another throughout most of their length, but nevertheless grown together by their adjacent edges firmly enough to form a weak tube. The vertical units are of course the six petals. Each is so wide that it rubs shoulders, so to speak, with each of its two neighbors, and, since all of them develop in unison, there is in a sense a unit growth of a cylinder. At the same time, however, each petal is completely surrounded by its own epidermis, and certainly possesses its own individuality. There is no difficulty in tracing the epidermal layers of adjacent petals up through the "joint" where they are in contact (fig. 32), excepting in the middle region of the tube where papillate epidermal cells of each petal invade intercellular intervals in the other (figs. 33, 34). At the upper end of the corolla tube the petals diverge not suddenly but very gradually from one another (fig. 35), each with papillate epidermal cells which correspond with epidermal intervals in the other. The corolla tube then is composed of members which, although adherent without being fused by cell fusions, are nevertheless distinct.

The epidermis of the petals is not cutinized. It bears hairs on both outer and inner surfaces, all hairs being simple and multicellular and probably glandular, although the terminal glandular cell in every case lacks the large granules which occur in the receptacle glands (fig. 36). The parenchyma is spongy throughout (fig. 31), and, for a short distance up from the receptacle, is chlorophyll-bearing. No stomata have been seen in the epidermis of petals.

Each petal is traversed by five vascular bundles, all of which enter from the receptacle. None of these bundles is branched excepting near the distal end of a petal, and even there branching is not frequent. Not far behind the petal tip each of the two marginal bundles connects with its nearest neighbor, which lies about midway between itself and the median bundle of the petal (fig. 42). This connection may be direct, in which case the marginal bundle itself anastomoses with its neighbor, or it may be indirect, in which case the marginal bundle is connected by a branch with the neighboring one. In the latter event the marginal bundle extends but little nearer the petal tip, where it ends blindly.

STAMENS

Soon after the rise of the three stamen primordia (fig. 8), meristematic activity is resumed in the periphery of the receptacle between those two which are nearest together, and from then on the two original primordia thus involved develop as a single bilobed organ (figs. 10, 12). The third primordium develops independently. It follows, therefore, that, although three stamens have their start in the embryonic flower, there will be only two distinguishable ones in the mature flower. It is obvious that the two mature organs cannot be homologous, for while one is a simple stamen, the other, being made up of two simple ones, is compound. It needs to be emphasized that the compound stamen has resulted not from a fusing of two separate members, but from two members which, possessing somewhat of their respective individualities, have grown up almost in unison without having ever been entirely separate.

After elongating slightly the stamens begin to lobe, developing at their free ends rounded protrusions which form right angles with their main axes (figs. 11, 13). As would be expected, the compound stamen develops twice as many main lobes as does the simple stamen (fig. 14). When seen in section, however, the subdivision of the main lobes will seem to vary, depending upon the vertical level from which the section is taken.

The two stamens maintain their separate identities for some time, but after a while even these are lost to the superficial observer; for as the stamens become thicker and as their inner faces are forced together throughout all but the basal part of their length, they come to have the appearance of one organ with two stalks (fig. 15). Since considerable growth and adjustment occur after such contact is effected, the two organs become strongly adherent to each other, papillate epidermal cells of the one dovetailing with similar cells of the other (fig. 37). Close as this union may be, however, no cases have been seen of cell fusions.

At about the time that the stamens begin to lobe the sporogenous tissue becomes differentiated. At first the subepidermal tissue to a depth of four or five cells is dense and deeply staining, and all of these cells have essentially the same appearance. Soon thereafter some of the cells of the outermost hypodermal layers cease to divide as rapidly as their neighbors and become conspicuously larger; thus differentiated, they constitute the archesporial tissue. This tissue occurs in two rows which represent the two microsporangia, and they are so arranged about the stamen as to describe a much compressed letter S. The small stamen develops but one of these double rows, or two sporangia, while the large stamen develops two double rows, or four sporangia (fig. 1). In the tetrasporangiate stamen each pair of S-shaped sporangia is so oriented that its lower end is near the flower center and its upper end is away from the center and toward the circumference, In the bisporangiate stamen the plane of the S is parallel with a cord of the floral circumference, and hence neither of its ends is nearer the flower center than the other.

All of the archesporial cells divide periclinally, each original S-shaped row thus forming two rows, one outer and one inner. The outer one is immediately beneath the stamen epidermis and is the primary parietal row, while the inner one is next inside the primary parietal row and is the primary sporogenous row. The primary parietal cells, by dividing longitudinally, transversely, and tangentially, develop a three-layered sporangium wall under the stamen epidermis (figs. 38–40), the innermost of which becomes the tapetum. While most of the primary sporogenous cells grow directly into large microspore mother cells without further division, a few divide once or twice; as a consequence the row of sporogenous cells, originally one cell thick throughout, becomes two or three cells thick here and there in its course. The mother cells round off and divide twice, each forming four microspores. The latter are held together for a time in a mucilaginous matrix which forms, apparently, from some of the mother cell cytoplasm that does not participate directly in forming the four spores.

As the stamens mature, their connectives become highly spongy by reason of the formation of large schizo-lysigenous cavities. The growth pressure to which the contiguous faces of the stamen connectives are subjected, plus the disintegration of subepidermal cells, together serve completely to obliterate, here and there, the visible boundary between the stamens as they are observed in section. However, throughout most of the boundary region the two limiting epidermal layers are distinct, even in mature stamens. And even if both epidermal layers cannot always be distinguished along the boundary, in most cases at least their location is marked by a sheet of collapsed and distorted cells which take a deeper stain than do the fragmentary neighboring tissues.

Each stamen, whether tetrasporangiate or bisporangiate, is traversed by a single bundle which enters from the receptacle. Neither bundle branches excepting near the distal end of the organ, but here the bundle in the small stamen has one branch which extends toward the coiled sporangia, while that in the large stamen has two branches (fig. 43).

VASCULAR CONTINUITY

The flower pedicel is traversed by three vascular bundles. Where the pedicel broadens out into the receptacle these bundles break up into numerous, short, anastomosing branches which form a low dome-shaped tangle of vascular elements. From this tangle, through which it is impossible to trace any single course, twelve bundles ascend obliquely through the receptacle and supply the perianth. As shown in fig. 41, however, a few of the twelve have indirect rather than direct connection with the vascular dome, for they are but branches from other bundles which do rise directly from the dome.

In the upper reaches of the receptacle cup six alternating bundles of the twelve are branched in a very definite manner (fig. 42). Each of the six branched bundles has two lateral (tangential) branches, beyond which it continues its course up and out into a sepal. Each of the two side branches continues out into a petal on the corresponding side. Just above the origins of these two branches they themselves are connected by another short bundle, oriented horizontally, and from the last there arise two more which extend out as marginal bundles into corresponding petals. The six bundles of the receptacle which do not branch before leaving it extend directly out into the six petals, each bundle occupying the median position. Each sepal therefore is supplied with a single bundle which either may or may not branch after leaving the receptacle. And each petal is supplied with five bundles, the middle one of which extends out directly from the receptacle base, right and left intermediate ones of which arise as branches from right and left sepal bundles respectively, and marginal ones of which arise as branches from the short, horizontal connecting bundle that unites the two branches of a sepal bundle.

Each stamen is supplied by a single bundle which extends upward directly from the vascular dome at the base of the receptacle (fig. 43). No constant association between these two stamen bundles and those supplying the perianth could be discerned, although such association does exist in the pistillate flower, as already described.

Discussion

Three matters having considerable morphological significance stand out prominently from the foregoing detail: (1) the unit individuality of the bisporangiate stamen; (2) the occurrence of stamens in two distinct cycles instead of one; and (3) the polypetalous condition of the corolla. Because of their significance, each of these three is worthy of some discussion; and all may be brought to bear finally upon the taxonomic position of the family.

BISPORANGIATE STAMEN

Three classes of facts lead to the conclusion that the bisporangiate stamen is in itself complete, and that the tetrasporangiate one is double. In the first place the tetrasporangiate stamen, as described for *Echinocystis*, has its origin in two primordia that are distinctly separate. BAILLON (1) describes exactly the same state of affairs for *Bryonia*, as does also PAYER (quoted by BAILLON). In these instances at least the larger stamen is double in its origin. Union of its two components, whatever the method of it, occurs subsequently and is incidental rather than fundamental. It is true that HEIMLICH's (6) figures of *Cucumis* do not show two primordia for each tetrasporangiate stamen, but of this fact there are two possible explanations, neither of which is contradictory to the present conclusion. It is possible that HEIMLICH's figures do not represent young enough primordia at the right cross-section level, or, if they do, it is possible that meristematic activity between the paired primordia begins so early in this species that the two are never visibly distinct.

In the second place the staminodium habit in pistillate flowers strongly suggests the double nature of the tetrasporangiate stamen. The pistillate flower of *Fevillea*, according to BAILLON, has five staminodia which correspond with five bisporangiate stamens in the staminate flower. *Bryonia* also has five staminodia in its pistillate flower, but only three staminate masses in the staminate flower. That the three masses are really the expression of five stamens is indicated, not only by the mere occurrence of five staminodia, but also by the fact that these five separate organs are arranged into three groups, corresponding with the three masses in the staminate flower. *Echinocystis* reveals a similar situation; two staminate masses (three stamens) in the one flower and three staminodia, of which two are paired, in the other. Since subsequent growth changes in the stamen representatives are less apt to occur in the pistillate than in the staminate flower where the stamens are functional, distribution evidence and individuality evidence come, in the former, to have considerable force. Some genera, it is true, have staminodia in one flower equal in number to the staminate masses in the other, even when two such masses are each tetrasporangiate. Such cases are worth investigating, for the small number of staminodia may have resulted from reduction in the androecium, from abortion of some of its members, or even from combined unit growth such as has been described between two functional stamens.

In the third place *Thladiantha*, as described by TISON (quoted by BAILLON), stands intermediate in position between the five-separate-stamen situation and the five-stamen-mass situation, and it clearly shows the homology between the two. *Thladiantha* has five bisporangiate stamens, all of which are separate, but four of them are arranged in two pairs whose members, although not in any sense united, stand close together. The fifth is isolated without a mate. Here then are five stamens in three groups; not very different from the situation in *Cucumis*.

NAUDIN (18) refuses to grant the bisporangiate stamen the status of a complete organ: (1) because there is no evidence of fusion on the filaments of tetrasporangiate stamens; (2) because, in perfect flowers, the stamen masses are equal in number to the carpels and in fixed relation of symmetry with them; and (3) because of a monstrosity seen in *Lagenaria*, where the bisporangiate stamen had grown another "half" which was green and leaflike, but whose undulating margin simulated the S-shaped sporangia of the normal organ. In answer to the first contention it may be repeated that the tetrasporangiate stamen is double, not because two separate members have fused together, but, as already shown in *Echinocystis* and as intimated by VAN TIEGHEM (24), because of the rise of a growing point between two primordia such as to involve both in the development of one single staminate mass. NAUDIN'S second contention cannot stand in the face of genera whose staminodia are isomerous with petals rather than with carpels, and the third one falls in the absence of an explanation for the monstrosity. Such a monstrosity may result from any one of a number of varied conditions, and it proves the half nature of the bisporangiate stamen no more than petalody proves the normal flower to be but half complete.

HEIMLICH'S agreement with NAUDIN seems to rest on (1) the absence of vascular evidence for any missing stamens or stamen parts; (2) the sporangium number which, in the small stamen, is just half the number that is common to most stamens; (3) the alternation of stamen masses and of staminodia with carpels; and (4) the equality in number between stamen primordia and mature stamens. Of course, if all five stamens are present in the flower, as I believe them to be, one could hardly expect to find vascular evidences of any missing ones. But even if some were missing there would likely be no vascular traces left for their identification, for vascular differentiation follows a growing point rather than precedes it; if there is no growing point there is small chance of there being any bundles. The absence of telltale traces then cannot be interpreted as evidence that three is the necessary stamen number in *Cucumis* and two in *Echinocystis*, nor is it evidence that there were never five stamens in the former and three in the latter. While agreeing with HEIMLICH that the number of bundles in an organ cannot be regarded as proof of its homology, I cannot refrain from mentioning the strong suggestion which the double bundle has in each tetrasporangiate stamen of *Cucumis* as contrasted with the single bundle in the bisporangiate stamen. Similarly the number of sporangia per staminate mass cannot be given much weight in settling homologies, for it is well known that variation exists in this respect among angiosperms. From what may be regarded as a single sporangium in the stamen of *Lemna* there is progression up to large numbers in the branched stamens of such forms as *Ricinus* and *Callothamnus*, and two sporangia per stamen is the normal situation in the Asclepiadaceae. Alternation of staminodia or stamen masses with carpels can hardly be significant, in view of the bundle associations already mentioned in the pistillate flower of *Echinocystis*, and in view of the numerous cases in which carpels and staminodia are not equal in number. Finally, the matter of numerical equality between stamen primordia and mature stamens has already been disposed of earlier in this paper.

On the basis of evidence at hand, therefore, it is my conviction that the bisporangiate stamen in Cucurbitaceae is a complete stamen; in no developmental sense at all is it a half organ. On this basis it is probable that *Telfairia* has ten stamens produced in five pairs; that *Fevillea* has five separate stamens; that *Thladiantha*, also with five, has its stamens in three groups; that *Cucumis* has five stamens, four of which are united in two masses; and that *Cyclanthera* has five stamens all united in one mass. *Echinocystis*, on a trimerous plan, has three stamens, two of which are united in one mass.

PENTACYCLIC SITUATION

The ten stamens of *Telfairia* furnish indirect evidence of two stamen cycles in that genus, while the present investigation furnishes direct evidence of the same situation in *Echinocystis*. If two stamen cycles are at all common in the family, it is anything but surprising that VAN TIEGHEM (24) and EICHLER (4) should disagree as early as they did over the question of stamen distribution, the former seeing stamens opposite the petals and the latter seeing them opposite the sepals. Nor is it strange that BAILLON (1) should see the single stamens opposite the sepals and the double ones opposite the petals. Further investigation is needed to determine how common this matter is, and also to determine whether or not combined stamens are always members of different cycles, as they apparently are in *Echinocystis*.

POLYPETALY

The idea of polypetaly in the Cucurbitaceae is by no means new, particularly to those taxonomists whose acquaintance with the family is not limited to our common North American forms. NAUDIN (18) goes so far as to say that only exceptionally are the petals fused. BAILLON (1) says of the group in general that they are clearly polypetalous, and he speaks also of five free petals in *Bryonia*. In BAILEY'S *Manual* and again in BRITTON and BROWN'S *Flora* the polypetalous condition is described for a part of the family, but it seems not to have been realized that our sympetalous genera too may be fundamentally polypetalous, as described for *Echinocystis*. HEIMLICH does not describe the corolla situation in *Cucumis*, and although he does mention vascular bundles which occupy positions exactly between the corolla lobes, even these would not exclude the possibility of petal individuality, since essentially the same thing is seen over and over again in the intercarpellary bundles of compound ovaries.

That polypetaly is more widespread in the family than is superficially apparent there is no doubt. Here again more investigation is needed, since it is the tubular corolla of the Cucurbitaceae that has been responsible for the inclusion of this family with the Sympetalae in the ENGLER and PRANTL system.

Conclusion

WERNHAM (25) has summarized the morphological characters of the Cucurbitaceae which have led to the inclusion of the family in the Sympetalae, and has adversely criticized each one. He mentions particularly the matter of cohesion in the androecium, that of the multilocular ovary, and that of the tubular corolla. But it is the adherence of anthers by their edges, as in Compositae and Lobeliaceae, that marks the evolutionary tendency in the upper sympetalous families, and this character is fundamentally different from the unison growth of stamen filaments and stamen connectives in the Cucurbitaceae. Likewise the multilocular ovary of the Sympetalae, divided up as it is by the radial walls of the several involved carpels, differs fundamentally from the cucurbitaceous ovary, which is basically unilocular, but which becomes multilocular secondarily by the enormous ingrowth of its parietal placentae. And finally the sympetalous character itself of the Sympetalae is fundamentally different from that of many, at least, of the Cucurbitaceae.

When to the foregoing there are added the facts that the Cucurbitaceae, unlike other Sympetalae, have ovules with two integuments and a large nucellus, and that, unlike other high Sympetalae, its flowers are prevailingly monoecious or dioecious, there can hardly be much surprise occasioned by WERNHAM'S expression, "Cucurbitaceae with somewhat doubtful affinity." In BENTHAM and HOOKER'S system, as also in WARMING'S, this family was included with the Archichlamydeae in spite of its apparently united petals. And now, with doubt cast on the sympetalous character of its flowers, there is no reason left for retaining the family in the group of sympetalous "foreigners."

The family, or at least a part of it, must also be degraded from its supposed tetracyclic position to the humbler pentacyclic one. There is evidence of variation in this character, but it would be expected anyway since the family is clearly a border-line one.

Summary

1. The order of appearance of organs in floral ontogeny is sepal, petal, stamen. Carpels are not represented.

2. The growth of floral organs is accomplished by means of both cell division and cell enlargement. Neither of these processes is restricted to a definite meristem or cambium.

3. Sepals are completely separate and therefore there is no calyx cup. Each sepal is traversed by a single bundle which enters from the receptacle.

4. While the petals form a loose corolla tube, each has its own epidermis which completely and distinctly marks it off from its adherent neighbors, and which clearly establishes its individuality.

5. Each petal is traversed by five bundles, all of which enter from the receptacle. All but the median bundle are derivatives of sepal bundles.

6. The completeness of the bisporangiate stamen is believed established by the fact that the tetrasporangiate stamen has its origin in two separate primordia; by the fact that staminodia in the pistillate flower, although disposed like stamen primordia, commonly develop without combining in pairs; and by the fact that *Telfairia*, with its stamens in an intermediate position between two extremes, clearly shows the homology between a tetrasporangiate stamen and two bisporangiate stamens.

7. Vascular associations between staminodia and perianth members in the pistillate flower indicate that the three stamens are members of two stamen cycles; two are opposite petals and one is opposite a sepal.

8. Each division of the androecium, whether bisporangiate or tetrasporangiate, is traversed by a single bundle. This bundle gives off one branch in the former and two in the latter. It is connected directly with the vascular anastomosis in the base of the receptacle rather than with bundles which supply the perianth.

9. With polypetaly known in the family there remains no longer a reason for including Cucurbitaceae in the Sympetalae. And with the pentacyclic condition known in some members, the family comes to take on more aspects of a highly variable, border-line group.

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EXPLANATION OF PLATES XIII–XVI

PLATE XIII

FIG. 1.—Diagram of androecium: *A*, habit showing face view of tetrasporangiate stamen; *B*, transverse section showing each pair of sporangia cut three times; *s*, bisporangiate stamen; *s'*, tetrasporangiate stamen.

FIG. 2.—Longitudinal section of flower primordium; ×200.

FIG. 3.—Longitudinal section of young flower with concave receptacle; ×200.

FIG. 4.—Longitudinal section of young flower with primordia of organs: *p*, petal; *s*, sepal; ×200.

FIG. 5.—Longitudinal section of young flower with stamen primordia appearing on floor of receptacle; ×200.

FIG. 6.—Longitudinal section of young flower with stamen primordia distinct and receptacle beginning to elongate; ×200.

FIG. 7.—Longitudinal section of young flower with petals and sepals carried above stamens on receptacle: *p*, petal; *r*, receptacle; *s*, sepal; *st*, stamen; ×200.

FIG. 8.—Transverse section of young flower at stage in fig. 6, showing three stamen primordia with two close together; ×200.

FIG. 9.—Transverse section of young flower at stage in fig. 7, and taken at petal level; ×200.

FIG. 10.—Longitudinal section of slightly older flower, showing lobed character of right-hand stamen; ×200.

PLATE XIV

FIG. 11.—Longitudinal section of flower showing early lobing of stamens: *p*, petal; *r*, receptacle; *s*, sepal; *st*, stamen; ×80.

FIG. 12.—Transverse section of flower at same stage as that in fig. 11, showing double nature of large stamen after establishment of growing point between two original primordia; ×80.

FIG. 13.—Longitudinal section of older flower with sporangia organized; note two stamen masses clearly separate; ×80.

FIG. 14.—Transverse section of flower at same stage as that in fig. 13: *m*, microsporangium; *s*, tetrasporangiate stamen; *s'*, bisporangiate stamen; *v*, vascular bundles; ×80.

FIG. 15.—Longitudinal section of older flower showing two stamen masses grown together above stalklike bases; ×52.

FIG. 16.—Cells from receptacle of fig. 6; ×410.

FIG. 17.—Cells from receptacle of fig. 7; ×410.

FIG. 18.—Cells from receptacle of fig. 15; ×410.

FIG. 19.—Cells from receptacle of mature flower; ×410.

FIG. 20.—Transverse section of receptacle cup of mature flower; ×275.

FIG. 21.—Section through stoma of mature receptacle; ×410.

FIG. 22.—Longitudinal section of glandular hair from upper and inner surface of receptacle; ×275.

FIG. 23.—Glandular hairs from lower and inner surface of receptacle: *A*, longitudinal section; *B*, *C*, transverse sections through terminal gland; ×275.

PLATE XV

FIG. 24.—Longitudinal section of hair from outer receptacle surface; ×275.

FIG. 25.—Cells from sepal of fig. 6; ×410.

FIG. 26.—Cells from sepal of fig. 7; ×410.

FIG. 27.—Cells from sepal of fig. 15; ×410.

FIG. 28.—Cells from petal of fig. 6; ×410.

FIG. 29.—Cells from petal of fig. 7; ×410.

FIG. 30.—Cells from petal of fig. 15; ×410.

FIG. 31.—Cells from petal of mature flower; ×410.

FIG. 32.—Transverse section through "joint" of corolla tube just above receptacle; dotted line marks boundary between petals; ×410.

FIG. 33.—Same as fig. 32, but about one-third up length of corolla tube; dotted line marks boundary between petals; ×410.

FIG. 34.—Same as fig. 32, but about half up length of corolla tube; here it is impossible to trace the two epidermal layers through "joint"; ×410.

PLATE XVI

FIG. 35.—Same as fig. 32, but up at level where petals are beginning to diverge from one another; intercellular spaces appear between the two epidermal layers; ×352.

FIG. 36.—Longitudinal section of young petal with epidermal hairs; ×352.

FIG. 37.—Transverse section through boundary region between bisporangiate and tetrasporangiate stamens: *c*, schizo-lysigenous cavities; *e*, epidermal layers of two stamens; ×235.

FIG. 38.—Transverse section of young anther with primary sporogenous cell that grows into spore mother cell: *e*, stamen epidermis; *p*, primary parietal layer; ×570.

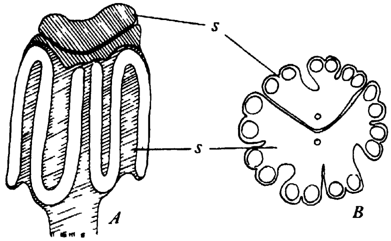
FIG. 39.—Transverse section of young anther whose sporangium wall is developing from primary parietal cells: *e*, stamen epidermis; *p*, parietal cells; ×570.

FIG. 40.—Transverse section of anther whose sporangium has developed three-layered wall: *t*, tapetum; ×570.

FIG. 41.—Diagram of round receptacle showing distribution of vascular bundles extending from basal, anastomosing tangle of short strands out to periphery where they will supply petals and sepals; not all twelve bundles connect directly with basal vascular mass.

FIG. 42.—Diagram of receptacle, petals, and sepals showing bundle distribution: *a*, anastomosing bundles at receptacle base; *p*, petal; *r*, receptacle; *s*, sepal.

FIG. 43.—Diagram of androecium showing bundle distribution: *b*, bisporangiate stamen; *t′*, tetrasporangiate stamen.



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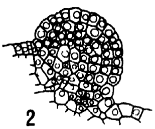

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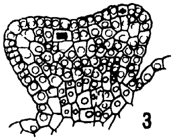

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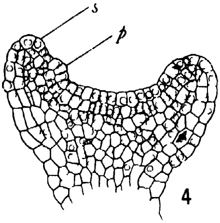

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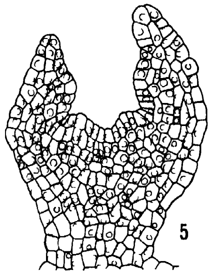

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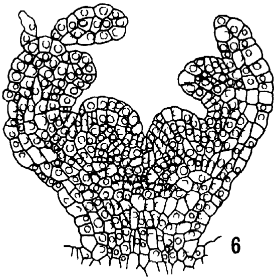

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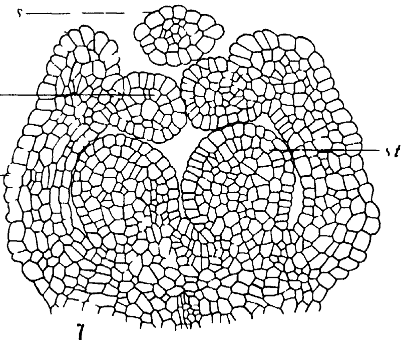

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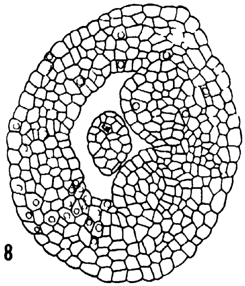

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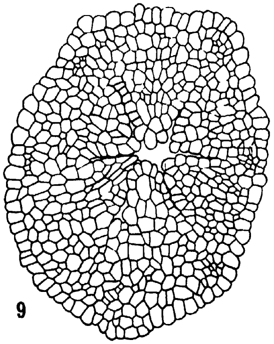

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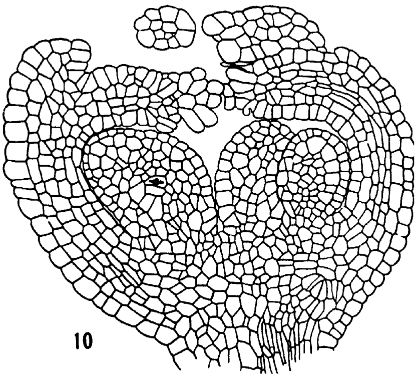

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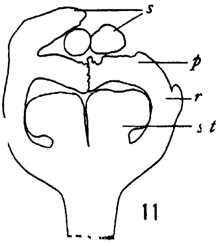
MILLER on ECHINOCYSTIS



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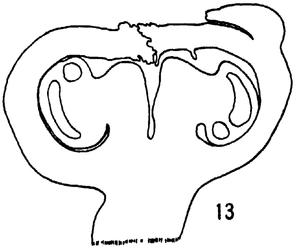

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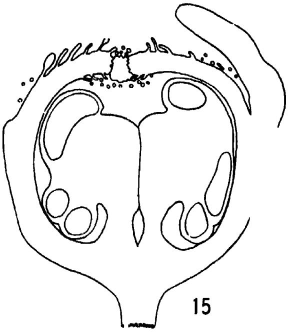

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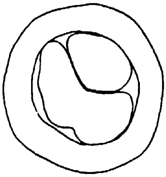

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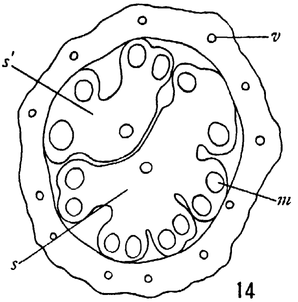

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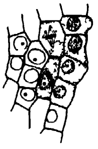

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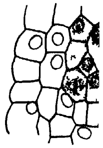

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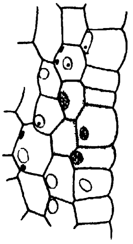

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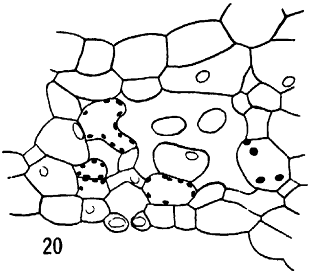

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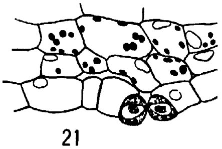

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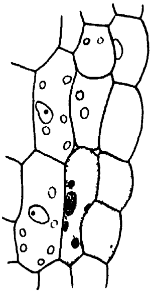

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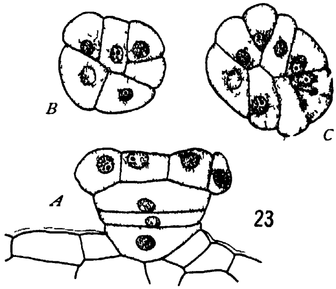

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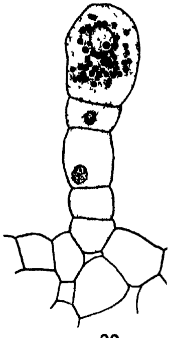

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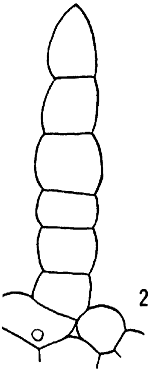
MILLER on ECHINOCYSTIS



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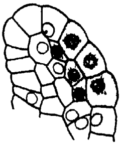

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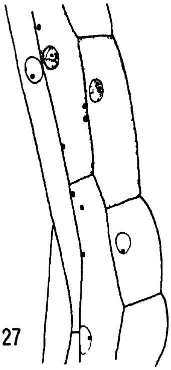

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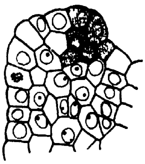

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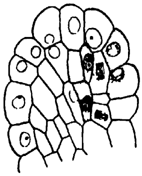

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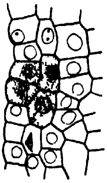

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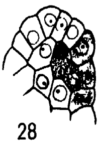

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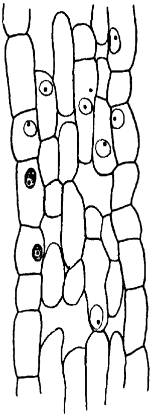

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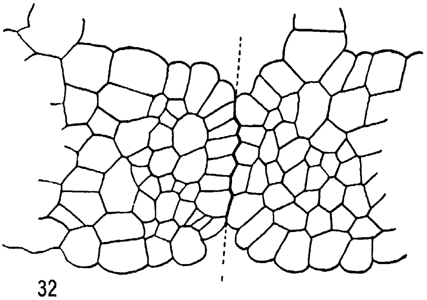

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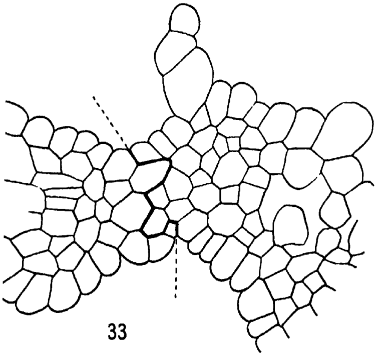

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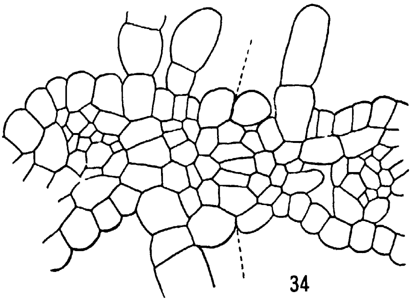

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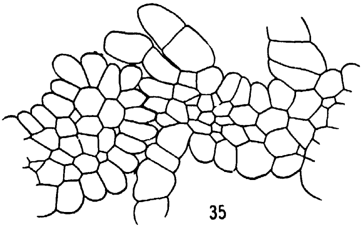

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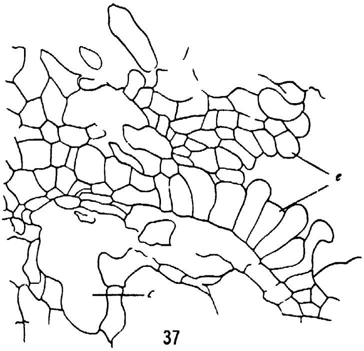

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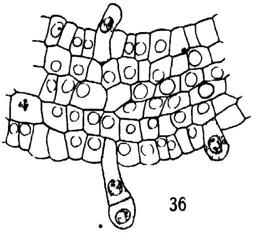

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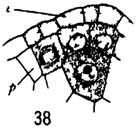

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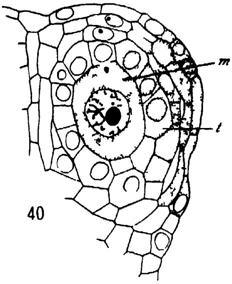

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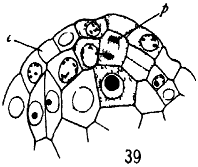

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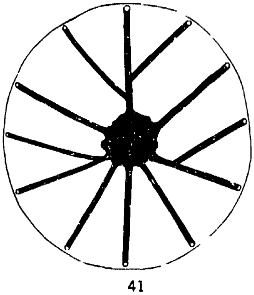

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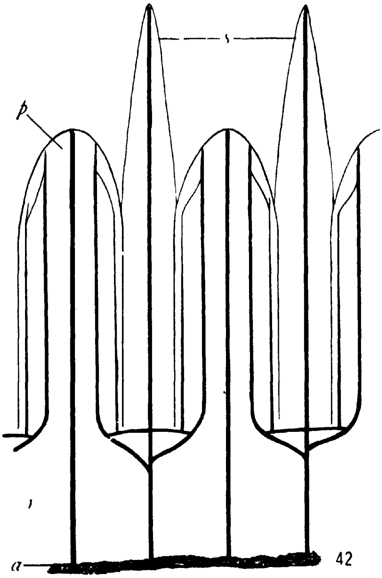

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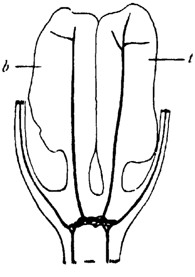

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MILLER on ECHINOCYSTIS

NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. III

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 397

EARL EDWARD SHERFF

(WITH PLATES XVII–XXI)

BIDENS PILOSA var. BIMUCRONATA f. ODORATA (Cav.) Sherff, BOT. GAZ. 81:41. 1926; *Cosmos tenellus* H. B. K. Nov. Gen. et Sp. 4:188 (240). 1820; *Bidens inermis* Wats. Proc. Amer. Acad. 23: 278. 1888. (pl. XVII)

To the list of synonyms for the forma *odorata* may be added also *Bidens inermis* Wats., a name founded upon *C. G. Pringle* 1291, in thin soil on rocky ledges, Arroyo Aucho, in the Sierra Madre, State of Chihuahua, Mexico, October 14, 1887 (type, Herb. Gray: cotypes, Herb. Berl.; Herb. Boiss.; Herb. Univ. Calif.; Herb. Field Mus.; Herb. Kew; Herb. Phila., etc.). *Pringle* 1638, 5999, and 7904 all are identical as to fruiting capitula with the type material of *B. inermis.* They all have the achenes especially slender and long-attenuate, as well as exaristate. The forma *odorata* is known, however, frequently to produce achenes wholly or in part of this very type, thus leaving no grounds for the retention of WATSON'S *B. inermis* as a species.[1] It may be remarked in passing that O. E. SCHULZ, in 1911, studied some of the *B. inermis* material (e.g., *Pringle* 1638) in the Berlin Herbarium and labeled it *B. caucalidea* DC., which in turn, as pointed out in my former article, is merely another synonym for f. *odorata.*

The type of *Cosmos tenellus* H. B. K. was collected between the City of Mexico and Huahuatoca (variously spelled Huehuetoca, Gueguetoque, etc.) in Mexico. A search by me in 1914 and again in 1924, among the type sheets of the HUMBOLDT, BONPLAND, and

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[1] The singular aspect of the fruiting heads upon typical "*B. inermis*" might be taken as justifying a *subformal* rank under f. *odorata*, but the already lengthy name *B. pilosa* var. *bimucronata* f. *odorata* would seem to suggest restraint in this matter. Furthermore, some material (e.g., *Dr. Edward Palmer* 673, Herb. Kew) has fruiting capitula precisely like those in the type collection of *B. inermis* but merely 3-5-partite leaves as in *B. pilosa* var. *bimucronata* proper, thus displaying an overlapping of characters.

KUNTH set at Paris, failed to reveal a specimen with the name *Cosmos tenellus;* nor was there authentic material at Berlin. The description by KUNTH shows that the type was without achenes. The bipinnatipartite foliage and the probably rosaceous color of the ligules evidently had led him to place the plant in *Cosmos*. A survey of *Cosmos* and *Bidens* at the present time indicates that KUNTH's extended description fits none other than f. *odorata* of *Bidens pilosa* var. *bimucronata* (Turcz.) O. E. Schz. This forma, as well as its supravening variety *bimucronata* (with which it frequently intergrades), is very abundant throughout the region surrounding the type locality of *Cosmos tenellus*. Moreover, the general aspect is ofttimes deceptively like that of *Cosmos*, a fact easily explaining KUNTH's reference of his type to that genus.[2]

BIDENS ANGUSTISSIMA H. B. K. Nov. Gen. et Sp. 4:183 (233). 1820; *B. angustifolia* H. B. K. *loc. cit.* 7:359 (456). 1825.—Occasionally references to this species are found in herbaria and libraries but with the spelling *angustifolia*. KUNTH's original description was under the name *angustissima*, a name that, from considerations of priority, must remain the valid one. The type was listed as growing "locis subfrigidis, prope Los Joares et Santa Rosa de la Sierra, alt. 1300 hex. . . . ." A search through the later volumes of KUNTH's work shows, in the 7th volume (*loc. cit.*) the name *B. angustifolia*. That the same species was meant is shown, however, by the context, "prope Los Joares et S. Rosa. (1300 h.)."

BIDENS CORIACEA (O. Hoffm.) Sherff, BOT. GAZ. 81:52. 1926; *Coreopsis coriacea* O. Hoffm., Engler Pflanzenw. Ost-Afr. 414. 1899; *C. ruwenzoriensis* S. L. Moore, Jour. Linn. Soc. 35:345. 1902; *Bidens ruwenzoriensis* (S. L. Moore) Sherff, BOT. GAZ. 59:309. 1915. (pl. XVIII)—In a former article (BOT. GAZ. 81:52. 1926) I pointed out the similarity between the types of *B. coriacea* and *B. ruwenzoriensis*, but was reluctant to merge the two into one species because of the lack of intermediate material. Since then I have studied specimens collected by *Sir Evan James*, Libu, Nandi Country, Uganda, British East Africa (Kew). These have the achenes *biaristate* as in the type of *B. ruwenzoriensis*, but the outermost bracts not *much longer* than the others. With this connecting form at hand, there appears no justification for attempting the further retention of *B. ruwenzoriensis* as a separate species.

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[2] It may be noted that several specimens collected by *Brother G. Arsène* in the States of Puebla and Michoacan (U. S. Nat. Herb.) have been distributed by *Arsène* under the name *Cosmos tenellus* H.B.K. and that these are the forma *odorata* of *B. pilosa* var. *bimucronata*.

BIDENS TRIPLINERVIA **nematoidea** var. nov.—A specie foliis numerosissimis et pinnatis vel bipinnatis, segmentis capillaribus plerumque tantum circ. 0.3–0.6 mm. latis differt.

H. L. Viereck 5, Cerro Quemado, Santa Marta, Colombia, Dec. 17, 1922 (type in U. S. Nat. Herb.); *Herbert H. Smith* 1980, rare on open lands, alt. 6500–7500 ft., San Lorenzo Ridge, Santa Marta, Colombia, Jan. 26, 1899 (Herb. N. Y. Bot. Gard.).

In 1913 the *Smith* plant from Santa Marta was noted by me as a singular foliage form and photographs (my photograph no. 341) were distributed to several herbaria. Recently I have been sent the *Viereck* plant, collected in the same locality and revealing similar characters. On both specimens the segments of the very numerous and pinnately or more often bipinnately parted leaves are strikingly threadlike. Otherwise the plants are like those of the very common var. *macrantha* (Wedd.) Sherff.[3]

BIDENS BIGELOVII **pueblensis** var. nov.—Folia principalia valde membranacea, tenuiter petiolata petiolis usque ad 3 cm. longis, petiolo adjecto 5–7 cm. longa, circumambitu triangulato-ovata, pinnata vel bipinnatisecta, segmentis primariis ovatis saepe 2–3 cm. longis et 1.2–1.6 cm. latis. Flores ligulati circ. 5, subflavi, tantum circ. 6–7 mm. longi. Achaenia inferne glabrata superne plus minusve erecto-setosa, apice biaristata aristis stramineis 1.5–3 mm. longis, retrorsum hamosis, dimorpha, exteriora clavata, badia vel rubrostraminea, corpore tantum circ. 4.5–5.5 mm. longa, interiora atra corpore usque ad 11 mm. longa et superne attenuata.

G. Arsène 7211, vicinity of Puebla, State of Puebla, Mexico, October, 1908 (type, U. S. Nat. Herb.); *idem* 5870, alt. 1950 m., Loma Santa Maria, vicinity of Morelia, State of Michoacan, Mexico, Sept. 4, 1910 (U. S. Nat. Herb.).

The foliage of *Arsène's* plants suggests strongly that of *Bidens duranginensis* Sherff, but the dimorphic achenes reveal the affinity with *B. bigelovii* Gray, a species which, in its typical form, is confined to a more northern range.

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[3] The capitula are 5-rayed; the mature achaenia (6–8 mm. long) are exaristate (as sometimes happens in the var. *macrantha* too) and are somewhat surpassed by the paleae. *Smith* reported the plants as growing erect and up to 18 inches high.

BIDENS TRIPARTITA **cernuaefolia** var. nov.—Folia indivisa vel summa subtripartita, breviter petiolata petiolis alatis circ. 1 cm. longis, petiolo adjecto 7-9 cm. longa, oblongo-lanceolata, membranacea, glabra, leviter serrulata, apice acuta sed parce acuminata, base sensim rotundata. Achaenia cuneato-oblanceolata, plana, purpurascenti-atra, faciebus glabra striataque, marginibus retrorsum hamosa, corpore 7-8 mm. longa et 1.5-2 mm. lata, apice biaristata aristis retrorsum hamosis 3-4 mm. longis.

R. C. Ching 4672, rare along open ditch of water, alt. 400 feet, Pei Chen, Province of Anhwei, China, Sept. 15, 1925 (type in Herb. Univ. Calif.).

Among the probably more than two thousand sheets of *Bidens tripartita* material studied by me, all specimens heretofore examined have proved referable either to the species proper or to one of three varieties, viz.: var. *hirta* (Jord.) Sherff, var. *orientalis* (Velen. *ex* Bornm.) Sherff, and var. *repens* (Don) Sherff. This plant, from the little known Province of Anhwei, appears to represent a variety quite distinct from any of the others. The leaves bear a strong resemblance to those found in some forms of *Bidens cernua* L. and of *B. laevis* (L.) B. S. P.

Bidens schizoglossa sp. nov.—Frutex ± 8 dm. altus, caule glabro, ramis subquadrangulatis subpurpurascentibus, glabris, internodiis quam foliis multo brevioribus. Folia tripartita, petiolata petiolis tenuibus eciliatis 1.5-3.5 cm. longis, petiolo adjecto 7-13 cm. longa, glabra, foliolis membranaceis, acriter dentibus terminaliter cuspidatis serratis vel (foliis summis) etiam integris, acuminatis, lateralibus lanceolatis et basaliter valde obliquis, sessilibus vel parce petiolulatis, 4-6 cm. longis et 1.5-2.5 cm. latis, terminali saepius ovato-lanceolato, oculis petiolulato, lamina 5-7 cm. longo et usque ad 4 cm. lato. Capitula numerosissima, corymboideo-paniculata pedicellis ultimis pubescentibus saepius tantum 2-4 mm. longis, radiata, pansa ad anthesin circ. 7-10 mm. lata et 3.5-5.5 mm. alta. Involucri pubescentis bracteae exteriores 4 vel 5, lineares, apice saepius subobtusae, tantum circ. 1.5 mm. longae, interiores lanceolatae circ. 2.5-3 mm. longae. Flores ligulati 5 vel etiam 6, flavidi, ligula obovati vel

saepe obdeltoidei, 3–4 mm. longi, apice moderate vel saepissime profunde perspicueque scissi, lobis 2 vel 3, terminaliter acutis subacutisve. Achaenia submatura linearia, plana, brunneo-atra, faciebus glabra et leviter paucistriata, marginibus parce subalata et inferne saepe setis elongatis erectis adnatis 1-3-setosis, sub vel fere ad apicem biaristata aristis calvis vel 1–3 hamis retrorsum hamosis, apice ipso plerumque erecto-setulosa, corpore ipso 7–8 mm. longa et circ. 1.2 mm. lata, quam paleis paulo breviora. (pl. XIX)

W. A. and *C. B. Setchell*, near Huehue, Island of Hawaii, Hawaiian Islands, June 24, 1924 (type, Herb. Univ. Calif., no. 247073).

A species closely allied with *Bidens micrantha* Gaud. and its varieties. It differs from that species and its varieties in outline or dimensions of the leaves, in having smaller and much more numerous capitula (and these upon shorter pedicels), in having the ligules often deeply and conspicuously cleft at the apex (whence "*schizoglossa*") and probably also in characters of the mature achenes, which last are lacking on the type. *B. ctenophylla* Sherff, the type of which (*Degener* and *Wiebke* 2128) came from the same locality, differs from *B. schizoglossa* in having different foliage, fewer and larger capitula, larger, differently shaped and entire or apically obscure-denticulate ligules, etc., although both species are similar in having minute, pubescent pedicels.

Bidens obtusiloba sp. nov.—Frutex glaber, ±6 dm. altus, ramosus ramis quadrangulatis, internodiis inferioribus quam foliis plerumque multo brevioribus. Folia tenuiter petiolata petiolis usque ad 3.5 cm. longis, petiolo adjecto usque ad 8 cm. longa, bipinnati-(plerumque biternati-) secta segmentis primariis circumambitu oblongo-ovatis vel saepe deltoideis, membranaceis, obsolete ciliatis, 1.5–3.5 cm. longis et paulo angustioribus, lateralibus breviter petiolulatis, omnibus in lobos vel dentes obtusos ac apice minute cuspidatos rursus dissectis. Capitula non numerosa, plus minusve corymbosa, radiata, pansa ad anthesin 1–1.5 cm. lata et 5–7 mm. alta, tenuiter pedicellatis pedicellis usque ad 1.8 cm. longis. Involucri nunc glabri nunc basaliter pubescentis bracteae exteriores 4 vel 5, lineares, apice subacutae, circ. 2 mm. longae, quam interiores lanceolatae dimidio breviores. Flores ligulati circ. 4 vel 5, flavidi, ±5 mm. longi, anguste obovati, apice 2- vel 3-dentati. Achaenia linearia, atro-brunnea, valde obcompressa, singula facie circ. 8-striata, duabus faciebus non nisi summam versus setosa, marginibus erecto-setosa, apice exaristata sed erecto-hispida, corpore 6–7.5 mm. longa et circ. 1 mm. lata. (pl. XX)

D. Le Roy Topping 2941, Niu Ridge, Island of Oahu, Hawaiian Islands, Nov. 30, 1924 (type, Herb. Univ. Calif. no. 305183).

Nearest apparently to ***Bidens pulchella*** (Less.) Schz. Bip., a species known only from its diminutive type specimen collected a century ago by *Chamisso* on the same island (*cf.* BOT. GAZ. **85**:25, pl. II, figs. *j-p*. 1928). The type of *B. pulchella* (Herb. Berl.) is a complete plant but only about 12 cm. tall, has the several principal leaves tripinnatisect with narrower segments than in *B. obtusiloba*, and has achenes distinctly clavate and only about 3 mm. long. Its aspect is that of an annual but this doubtless is due to the fact that the plant had been gathered in its first year, whereas the type of *B. obtusiloba* is a branch coming from a well developed shrub. A study of *B. obtusiloba* in its juvenile stages is much to be desired.

Bidens amphicarpa sp. nov.—Herba annua, gracilis, ramosa, 1–6 dm. alta, caule quadrangulato ac saepe purpurascenti, nunc pubescenti nunc glabrato. Folia tenuiter petiolata petiolis usque ad 2 cm. longis, petiolo adjecto usque ad 5 cm. longa, pinnatim 3–5-partita, foliolis valde membranaceis, glabratis vel sparsim adpresso-hispidis, ciliatis et plerumque minutissime atromarginatis, unico latere grosse 1–4-serratis dentibus acerrime apiculatis, lateralibus ovatis, terminali lanceolato vel fere lineari. Capitula in pedunculis tenuissimis usque ad 1 dm. longis ramos terminantibus disposita, radiata, pansa ad anthesin tantum circ. 6–7 mm. alta et 8–10 mm. lata. Involucri bracteae exteriores 5–8, tenuiter lineares, basaliter tergo margineque superne tantum marginaliter hispidae, apice acerrime mucronatae, demum circ. 4 mm. longae, interioribus lanceolatis atrobrunneis vel atropurpurascentibus sed marginaliter albido-diaphanis apicaliter ciliatis plerumque subaequalia. Flores ligulati circ. 5, ligula oblanceolati, 3–5 striis percursi, subflavidi vel subrosacei, apice saepe 2-(3-)dentati, 5–6 mm. longi. Achaenia exteriora subplana, unica facie circ. 4-sulcata, infra parce supra valde erecto-setosa, badia vel rubro-straminea, corpore 5–8 mm. longa, interiora

tereti-quadrangulata, omnino 8-sulcata, maximam partem atra sed apicaliter brunneo-straminea, erecto-setosa, elongato-linearia et supra saepe cervicem formantia, corpore demum 9–15 mm. longa; omnia recta vel subrecta 2–3-aristata aristis tenuibus retrorsum hamosis, 2.5–4 mm. longis, demum saepe caducis.

T. S. Brandegee, Sierra de Laguna, Lower California, Jan. 23, 1899 (type in Herb. Univ. Calif., no. 134269).

Differs from glabrous or subglabrous forms of the similarly amphicarpous *Bidens anthriscoides* DC. in the dissection and outline of the leaflets, in its proportionately much longer external involucral bracts,[4] and in its straighter, less hispid, and never tuberculate achenes. The general aspect is somewhat like that in stunted, slender forms of *B. pilosa* var. *bimucronata* f. *odorata* (Cav.) Sherff, but the very narrow external bracts, the reddish outer achenes, the dimensions of the flowering and fruiting heads (the latter with fewer achenes) all distinguish it from that form.

BIDENS SUBALTERNANS **simulans** var. nov.—A specie foliis plus dissectis, segmentis plerumque plus minusve linearibus, differt.

P. Dusén, in cultivated places, Tlapcrussú, State of Paraná, Brazil, Feb. 29, 1912 (U. S. Nat. Herb.); *idem* 9432, in grassy, shrubby places, State of Paraná, Mar. 25, 1910 (Herb. Gray; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *Dr. Wilhelm Herter* 787, alt. 30 m., clay soil, along roads, Toledo, Department of Canelones, Uruguay, May, 1927 (Herb. Gray; Herb. U. S. Nat.); *P. Jörgensen* 1283, Department of Andalgalá, Province of Catamarca, Argentina, Oct. 11, 1916 (Herb. Gray; Herb. U. S. Nat.); *idem* 1785, growing erect, 1 m. high, *eodem loco*, Oct. 1, 1917 (type, U. S. Nat. Herb.: cotype, Herb. Gray); *Dr. Otto Kuntze*, Province of Cordoba, Argentina, December, 1894 (Herb. N. Y. Bot. Gard.).

At times, *Bidens subalternans* DC. is found with the leaves delicately 2–3-pinnatisect and the segments linear, even narrowly so. Heretofore, when making herbarium determinations, I have included such forms under the species proper without distinction. In some cases, they suggest *B. bipinnata* L. so strongly as to create confusion.[5] In other cases, they display a slight approach to *B. exigua* Sherff.

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[4] In *B. anthriscoides* DC. the outer bracts are only about one-half or three-fifths the length of the inner ones, notwithstanding DECANDOLLE's original description ("invol. squamis . . . . inter se subaequalibus;" Prodr. 5:601. 1836).

[5] *B. platensis* Mang., described as a hybrid (An. Mus. Nac. Buenos Aires **24**:230. 1913) between *B. bipinnata* L. (pistillate) and *B. pilosa* L. (staminate), should be compared with this variety.

BIDENS SUBALTERNANS **unipinnata** var. nov.—A specie foliis pinnatim 3–5-partitis foliolis lanceolatis vel ovato-lanceolatis differt.

Dr. Emil Hassler 11558, in the region of Lake Ypacaray, central Paraguay, February, 1913 (type in Herb. Gray); *L. R. Parodi* 7787, adventive in cultivations, Lanugasta, Chilecito, Province of La Rioja, Argentina, Jan. 31, 1927 (Herb. Gray).

Occasionally a South American specimen of *Bidens* is found with the general aspect of *B. pilosa* L., yet with the fruiting heads as in the closely related *B. subalternans* DC. (*Hassler* 11558 and *Parodi* 7787 are examples). A close inspection shows that the only difference from typical *B. subalternans* is in the once-pinnate (instead of twice-pinnate) leaves.

Bidens glabrata (Gray) comb. nov.; *B. lantanoides* var.? *glabrata* Gray, Proc. Amer. Acad. **5**:128. 1861.—The type material of *Bidens lantanoides* Gray came from the Island of Eimeo (known also as Morea), of the Society Islands. It was collected by the *United States Southern Pacific Exploring Expedition* under *Captain Wilkes*. In a former article (BOT. GAZ. **85**:pl. V. 1928) I have illustrated it.

On the same expedition two small branches of another shrub were collected at the Island of Tahiti of the same island group. These are preserved in the United States National Herbarium. They are the types of GRAY's doubtfully advanced "var.? *glabrata*." While very fragmentary as to capitula, they nevertheless are complete enough to reveal important specific differences from specimens of *B. lantanoides* proper and indeed from any other species of *Bidens*. As they appear fully worthy to rank as separate species and since GRAY's description was much too brief, they were recently restudied by me and the following amplified description obtained:

Frutex, ramis subtetragonis, hinc inde hispidulis, minute striatis. Folia breviter ac late petiolata petiolis basaliter ciliatis circ. 1 cm. longis, petiolo adjecto 7–10 cm. longa et 2–3 cm. lata, oblonga, basaliter sensim apicaliter subabrupte attenuata, apice breviter acuminata, lamina glabra, crassiuscula, acriter serrata unico lateri 11–19 dentibus. Capitula corymboideo-paniculata, pedunculata pedunculis validis usque ad 8 cm. longis, verisimiliter radiata, involucris demum basaliter ±7 mm. latis. Involucri bracteae exteriores circ. 7, late lineares, apice subobtusae, tergo non pubescentes nisi basim versus, circ. 4 mm. longae; interiores oblongo-lanceolatae, 6–7 mm. longae. Achaenia submatura brunnea, matura atra, valde obcompressa, linearia, nunc inferne nunc superne attenuata, glabra, exalata, unica facie circ. 4-sulcata, corpore 4–5 mm. longa et circ. 0.8 mm. lata, apice biaristata aristis tenuibus retrorsum hamosis usque ad 2.2 mm. longis.

BIDENS TENERA **tetracera** var. nov.—Capitula discoidea ad anthesin 4–5 mm. alta et pariter lata. Involucri bracteae exteriores circ. 7 vel 8, lineares, tergo fere glabrae, margine ciliatae, sub apice interdum subdilatatae, apice ipso subacutae, circ. 3 mm. longae; interiores lanceolatae, 4–5 mm. longae. Achaenia linearia 15–30 in unico capitulo, fere usque ad apicem atra, apice ipso straminea, corpore exalata tetragona ac 1–1.5 cm. longa, circ. 0.5–0.65 mm. crassa, superne sensim attenuata, glabra, unica facie (4 facierum) 2-sulcata, apice quadriaristata aristis tenuibus, 2–3 mm. longis, retrorsum hamis elongatis tenuibus albidis hamosis.

Dr. Otto Buchtien 4182, at altitude of 1300 meters, Milluguaya, North Yungas, Bolivia, December, 1917 (type in U. S. Nat. Herb.); *H. Pittier* 10222, alt. 800–1000 m., around Caracas, Venezuela, Mar. 10, 1922 (Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

The type of *Bidens tenera* O. E. Schulz was collected in Costa Rica. At least twelve additional collections have been made, ranging southward and thence eastward into Colombia, Venezuela, French Guiana, and Brazil. At times, the species grows to a height of 5 or 6 decimeters, has tripartite leaves, produces as many as 20 achenes to a head, and then approaches the *Buchtien* plant from Bolivia. The latter, however, is still quite distinct in its more numerous achenes. These, moreover, are quadriaristate and upwardly attenuated, not triaristate and almost parallel-sided throughout as in the species' type. The general aspect is that of a thin-leaved form of *B. pilosa* L. as to foliage and of *B. bipinnata* L. as to fruiting heads.

Bidens biternata (Lour.) Merrill and Sherff, comb. nov.; *Coreopsis biternata* Lour. Fl. Cochinch. edit. I: 508. 1790; *ibid.* edit. II: 622. 1793; *Bidens chinensis* Willd. Sp. Pl. 3: 1719. 1804; *Actinea biternata* (Lour.) Spreng. Syst. 3: 474. 1826.—O. E. SCHULZ, in his special study of *Bidens chinensis* Willd. and related species (Engler Bot. Jahrb. 50 [Supplem.]: 178. 1914), appears to have overlooked the

Coreopsis biternata of LOUREIRO, published with a description in 1790, some fourteen years earlier than the date of WILLDENOW'S work. In view of the many difficulties involved in the interpretation of some of Loureiro's species, I have refrained until recently from attempting final disposition of his names. At last, however, these difficulties have been overcome and a certain and conclusive treatment becomes possible.

LOUREIRO described two species of *Bidens*, namely *B. pilosa* L. and *B. bipinnata* L., and two of *Coreopsis*, namely *C. leucorrhiza* Lour. and *C. biternata* Lour. The first two may be passed over here, since they were admittedly not new species and since, moreover, the known occurrence of these two species in the region mentioned by LOUREIRO (Cochin China and China) tends to confirm the identity of the LOUREIRO plants. The third species, *Coreopsis leucorrhiza* Lour., has recently been referred by me (BOT. GAZ. **86**:443. 1928) to *Bidens pilosa* var. *minor* (Bl.) Sherff.

The fourth species, *C. biternata* Lour., was known to LOUREIRO under the Annamese dialectic name *Ca ap chioc*,[6] and he stated that it grew in fields near Canton, China. His description follows: "Differ. spec. Cor. foliis biternatis, ovato-lanceolatis, serratis: panicula diffusa: radio sexfloro. Habitus et notae. Caulis herbaceus, 3-pedalis, erectus, 4-gonus, 4-sulcatus, integre luteus: panicula sparsa, terminali. Radii corollae 6, neutrae. Pappus bicornis, ramosus. Receptaculum planisculum, nudum."

His character for the foliage, *biternatis*, shows at once that he was dealing with the same plant as WILLDENOW treated under the name *Bidens chinensis*. His "*pappus bicornis*" was doubtless merely one of the numerous errors for which his descriptions were noted.[7] His "receptaculum .... nudum" probably was based upon capitula that had shed their achenes and chaff scales, for just previously, in his generic description of *Coreopsis*, he had said "recept. paleaceum."

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[6] LOUREIRO "lived at Hue for approximately thirty-five years, this town being the ancient capital of the kingdom of Cochin China, now a part of French Indo-China. I assume that the limits of the old kingdom of Cochin China were approximately the limits of the Province of Annam [Anam] to-day, in French Indo-China. On leaving Hue, Loureiro proceeded to Canton and spent two or three years there before proceeding to Lisbon. . . . . Loureiro tried to indicate in his native names as between Annamese (indicated by the letter "a") and Chinese names (really in Mandarin) by the letter "b" . . . . by China he means the general vicinity of Canton in Kwangtung Province. . . . . Generally speaking: I am of the opinion that most of his work was done on Cochin China specimens, chiefly for the reason that he resided so long in Cochin China and for a comparatively brief time in Canton; and the conditions in Canton at the time of his visit were such that it would have been impossible for him to visit any regions outside of the immediate vicinity of the city." . . . . DR. ELMER D. MERRILL *in litt.* April 22, 1929.

In my monographic study of *Bidens*, I have found a considerable number of specimens from the general region traversed by LOUREIRO and which may be cited in confirmation of his description.[8] A few of these are:

Mr. Balansa 910, near Quang-yen (Kwangyen), Tongking, (French) Indo-China, August, 1885 (Herb. Par.); *R. P. Bodinier*, Hongkong, China (Herb. Par.); *Eberhart* 2568, Hoi-mit, Indo-China (Herb. Par.); *idem* 3338 *bis*, Thanmoi (Than Muoi), Province of Langson, Cochin China (Herb. Par.; *nom. indig.* Cay nu ao); *F. Evrard* 154, near Saigon, Cochin China, Oct. 21, 1920 (Herb. Par.); *idem* 225, Dalat, Indo-China (Herb. Par.); *Gaudichaud*, Macao, Province of Kwang-tung, China, 1836–1837 (Herb. Par.); *A. Germain* 109, Cochin China (Herb. Par.); *G. W. Groff*, around fruit trees, Sun Ooi, Lai Ngok Village, Canton Delta, Kwang-tung Province, China, Mar. 18, 1918 (Herb. Univ. Calif.; *nom. incolarum* Kam p'un ngan chan); *Hana* 298, Hongkong, China (Herb. Gray); *Dr. Harmand*, Iles de Poulo-Condor, Cochin China, 1875–1877 (Herb. Par.); *Dr. Aug. Henry* 8269, Isl. of Hainan, China, November, 1889 (Herb. Berl., 2 sheets); *H. Lecompte* and *A. Finet* 1252, Barka, Indo-China, November, 1911 (Herb. Par.); *iidem* 1733, Angkor Thom, Cambodia, Indo-China, Dec. 12, 1911 (Herb. Par.); *E. Lefèvre* 4, Cochin China, Sept. 25, 1864 (Herb. Par.); *F. A. McClure*, grassy field, Kingchow, Isl. of Hainan, China, Oct. 14, 1921 (Herb. Univ. Calif.); *idem*, roadside near Kingchow, Isl. of Hainan, Apr. 5, 1922 (Herb. Univ. Calif.); *Elmer D. Merrill*, bamboo thicket and border of dry thicket, Honam Isl., Canton, Kwang-tung Province, China, Oct. 13–Nov. 9, 1916 (Herb. Univ. Calif., 2 sheets: "this is the very common form at Canton. Honam Island is directly in front of the city, across the river. E.M."); *Mr. Petelot* 1211, roadsides, Hanoi Viuh, Cho Ganh, Tonking, Indo-China, November, 1922 (Herb. Par.) and November, 1923 (Herb. Univ. Calif., 2 sheets); *Dr. Talmy*, Cochin China, October, 1867 (Herb. Par.); *Dr. Thorel* 1270, Nareiager, Cochin China, 1862–1866 (Herb. Par., *quo pro Loureirone nominata); idem* (*similiter*) 1270, Bassac (Bassak), Cambodia, 1866–1868 (Herb. Par., 3 sheets).

Recently I was supplied by DR. ELMER D. MERRILL, Dean of The College of Agriculture of The University of California, with an excerpt from page 553 of the as yet unpublished manuscript of his "A Commentary on LOUREIRO's Flora Cochinchinensis." It was interesting to find that MERRILL, too, had concluded *Coreopsis biternata* Lour. to be the same as *Bidens chinensis* Willd. Since then, he has very kindly assisted me with various desired data and now joins me in publishing the new combination which this conclusion makes necessary.

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[7] Occasionally the pappus is *tricornis* and it may very well be that he had so spelled the word on sending it to the printer for the first edition. In any case, however, this error did not get corrected in the second edition.

[8] These mostly bear my herbarium determination, *Bidens chinensis* Willd. or (L.) Willd.

BIDENS BITERNATA var. **abyssinica** (Schz. Bip.) comb. nov.; *B. abyssinica* Schz. Bip. *in* Walpers Repert. **6**:167. 1846–1847; *B. chinensis* var. *abyssinica* (Schz. Bip.) O. E. Schz., Engler Bot. Jahrb. **50** (Supplem.): 180. 1914.—This variety has been found, though rarely, as far northward as Arabia, British East India, Ceylon, the Provinces of Kiangsu and Shan-tung, China, and Corea. It is unknown, however, from French Indo-China or from Kwang-tung Province in southern China, in one or both of which places LOUREIRO obtained his type materials of *B. biternata* proper.

BIDENS SANDVICENSIS **typica** var. nov.; *B. sandvicensis* Less. *pro specie*, Linnaea **6**:508. 1831.

BIDENS SANDVICENSIS var. TYPICA **compositior** Deg. and Sherff, f. nov.—A var. *typica* foliis principalibus bipinnatisectis vel (saepe ternatim) bipinnatis differt. Achaenia biaristata aristis usque ad 1 mm. longis vel demum calvis.

Otto Degener and *Kazuto Nitta* 3411*a*, moderately dry, open slope at alt. 1500 feet, east rim of Manoa Valley, one mile mauka of the University, Isl. Oahu, Hawaiian Isls., Jan. 13, 1929 (type in Herb. Field Mus.); *iidem* 3412*a*, much windswept, sparingly wooded slope at alt. 2000 feet, east rim of Manoa Valley, one and one half mile mauka of University, Isl. of Oahu, Jan. 13, 1929 (Herb. Field Mus.).

Of twenty-seven collections of *B. sandvicensis* studied by me thus far, those by *Degener* and *Nitta*, nos. 3411 and 3412, were the first to be accompanied by a bipinnately leaved form. In each case only one specimen of this form had been found. The appearance of an entire plant is rendered strikingly unique by the dissection of the foliage. PROFESSOR DEGENER states that "apparently the more exposed to wind, the more parted are the leaves." In the case of the several dozen specimens sent me, however, the transition from the pinnate to the bipinnatisect or bipinnate type of leaves is very abrupt.

Greenmania sect. *Bidentis* nov.—Herbae Americae tropicae perennes, plerumque scandentes, caulibus saepe 5–10 m. longis; capitulis numerosis, normaliter radiatis; achaeniis elongatis, valde obcompressis vel omnino planis, lateribus parallelis et saepissime valde setosis, apice plerumque biaristatis aristis saepius longis nunc retrorsum hamosis nunc calvis.—**Type species,** *Bidens squarrosa* H. B. K.

The section includes a number of climbing species (*B. squarrosa* H. B. K., *B. rubifolia* H. B. K., *B. reptans* (L.) G. Don, *B. urophylla* Sherff, *B. speciosa* Gardn., etc.). DeCandolle (Prodr. 5:596–599. 1836) employed the section *Psilocarpaea* for many very unlike species, merging the climbers with such species as *B. pilosa* L. and *B. andicola* H. B. K. Various considerations as to restricted geographic distribution, scandent stem-habit, clustered arrangement of capitula, shape of achenial body (flat, parallel-sided, usually hispid upon the margins, commonly biaristate, in appearance often suggesting a centipede), indicate the advisability of designating the climbing species under a distinct *sectional* name.[9]

BIDENS PILOSA var. RADIATA **indivisa** f. nov.—A varietate ipsa foliis indivisis ovato-lanceolatisque differt.

J. S. De La Cruz 1792, growing 3 ft. high, Upper Rupununi River, near Dadanawa, Lat. 2°45′ N., British Guiana, Jul. 24–29, 1922 (type, Herb. Field Mus.); *idem* 2284, growing 2 ft. high, Upper Mazaruni River, Long. about 60°10′ W., British Guiana, Sept. 22–Oct. 6, 1922 (Herb. Field Mus.).

In his Flora of The British West Indian Islands (p. 373. 1861), GRISEBACH stated that "a remarkable form, with all leaves simple, hispidulous achenia, and whitish-pubescent involucres, was collected by Dr. Alexander, but transitions into the common *B. leucanthus* [*i.e., B. pilosa* var. *radiata* Schz. Bip.] occur among Mr. March's specimens." A former study of *Alexander's* specimens (Herb. Gray) left me unconvinced as to the value of their simple-leaved character for drawing taxonomic distinctions. The specimens recently collected by *De La Cruz* were obtained at different localities and about two months apart, yet both reveal in all respects a striking similarity.

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[9] It is a pleasure to use here the name *Greenmania,* thus expressing, although all too inadequately, my very great indebtedness to my former teacher, DR. JESSE MORE GREENMAN. He it was who suggested, in 1912, that I study the genus *Bidens* and who rendered invaluable assistance to me during the initial stages of my research, particularly upon certain species and varieties of this section.

The leaves all are simple, thin, glabrous, the blades ovate-lanceolate, serrate, acute or subacuminate at apex, the larger ones about 3.5–4 cm. long and 1.5–2 cm. wide, the petioles slender and 1–2 cm. long. Compared with chance, more or less simple-leaved plants of *B. pilosa* proper that are sometimes found (*B. pilosa β discodea* Schz. Bip. *em.* I. *subsimplicifolia* O. Ktze. Rev. Gen. 1:322. 1891; "folia omnia vel plurima integra [cfr. Gris. Fl. Westindien]"-Ktze. *loc. cit.*) these plants appear to represent a more pronounced and less ephemeral form.

BIDENS OSTRUTHIOIDES var. **costaricensis** (Benth. *ex* Oerst.) comb. nov.; *B. costaricensis* Benth. *ex* Oerst., Kjoeb. Vidensk. Meddel. 1852: 94. 1852; *B. irazuensis* Calv. and Calv. Year Costa Rican Nat. Hist. xvi, 137 (plate), and 140. 1917. (pl. XXI) The well known *Bidens ostruthioides* (DC.) Schz. Bip. (*B. guatemalensis* Klatt) has "folia petiolata petiolis inferne hispido-ciliatis 1.5–2.5 cm. longis, petiolo adjecto plerumque subaequaliter 4.5–6.5 cm. longa, membranacea, infra multo pallidiora, tripartita (vel saepe summa indivisa); foliolis ovatis vel rhomboideo-ovatis, ciliatis, utrinque acute grosseque dentato-serratis; dentibus utroque latere 1–5, indurato-mucronatis."[10] A study of the type and other specimens of *B. costaricensis* shows the "folia petiolata petiolis inferne hispido-ciliatis 1–4 cm. longis, petiolo adjecto 5–12 cm. longa, acerrime bipinnatisecta vel plus minusve biternatisecta, foliolis segmentisve cuneato-lanceolatis, inciso-dentatis, dentium apice indurato-apiculatis et saepe mucronatis, margine sparsim ciliatis; supra ad venas plerumque minutissime creberrimeque glanduloso-setulosis, aliter glabris, in speciminibus exsiccatis saepe nigrescentibus; infra pallidioribus; lateralibus decurrentibus." (See footnote 10.)

In flower and fruit characters the two are identical. In size of plants, *B. costaricensis* may become taller, since *Lehmann* found it climbing to a height of 5 m., whereas *B. ostruthioides* has been reported several times as growing 0.3–1.5 m. high. Geographically, the former is more southern (growing, nevertheless, mostly at lower altitudes), ranging from the State of Oaxaca, Mexico, to Costa Rica,[11] while the latter ranges from the three Mexican States of

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[10] *Ex meo chirographo ined.*

[11] Specimens of *B. ostruthioides* by *C. J. Graham* (Herb. Kew) from the region of the States of Mexico and Hidalgo (*fide* Benth. Pl. Hartweg. Pref. iv. 1839) exhibit, however, a strong approach in leaf division to *B. costaricensis*.

Michoacan, Mexico, and Vera Cruz to Guatemala. In Oaxaca both forms are common. Curiously, however, the *B. costaricensis* form, when found in Oaxaca, has been labeled at Gray Herbarium (*Pringle* 5848), the University of California Herbarium (*Purpus* 3109), and elsewhere as *B. ostruthioides*, while identical plants from Costa Rica have been referred very uniformly to *B. costaricensis*. A comparative study of all available material[12] indicates that *B. costaricensis* is best regarded as a variety of *B. ostruthioides*, from which it differs essentially in having the leaves somewhat larger and bipinnatisect or biternatisect, not merely tripartite.

Specimens examined: *Dr* and *Mrs. P. P. Calvert*, forest below cinders, El Volcan Irazú, Costa Rica, Apr. 2, 1910 (Herb. Univ. Penn.); *C. W. Dodge* 3439, in the oak forest on the upper slopes, El Volcan Irazú, Cartago Province, Costa Rica, Aug. 18, 1925 (Herb. Gray); *C. Hoffmann* 105, higher mountain forest of El Volcan Irazú, May 6, 1855 (Herb. Berl.); *Dr. Otto Kuntze*, alt. 7000 ft., Cartago, Costa Rica, June 24, 1874 (Herb. N. Y. Bot. Gard.); *F. C. Lehmann* 119, alt. 6500 ft., climbing to 5 m. high, among shrubs and bamboos, west slopes of El Volcan Irazú, Mar. 28, 1878 (Herb. Mus. Vienna);[13] *idem* 1787, alt. 800 m., growing up to 1.5 m. high, in moist places, Rio Blanco, Costa Rica, Mar. 18, 1882 (Herb. Boiss.; Herb. Gray; Herb. Kew, 2 sheets); *Anders S. Oersted*, alt. 2000–5000 ft., Mt. Aguacate, Costa Rica, November, 1846 (type, Herb. Copenh.); *idem* San José, Costa Rica, 1845–1848 (Herb. Copenh., 2 sheets); *idem*, El Volcan Irazú etc., Costa Rica (*ex herb. Benth. in* Herb. Kew); *H. Pittier* 742, alt. 2800–3200 m., in oak forests of El Volcan Irazú, Dec. 12, 1888 (Herb. Gray); *idem* 14070, alt. 2300 m., Laguna del Reventado, El Volcan Irazú, Jan. 1, 1901 (Herb. Gray); *C. G. Pringle* 5848, alt. 9000 ft., Sierra de Clavellinas, State of Oaxaca, Oct. 26, 1894 (Herb. Gray); *C. A. Purpus* 3109, Cerro Verde, State of Oaxaca (in vicinity of San Luis Tultitlanapa, Puebla, near Oaxaca), July, 1908 (Herb. Berl.; Herb. Brit. Mus. Nat. Hist.; Herb. Univ. Calif.); *Charles L. Smith* 357, alt. 9000 ft., Sierra de Clavellinas, State of Oaxaca, Oct. 16–19, 1894 (Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

Coreopsis intermedia sp. nov.—Herba erecta, plus minusve glabra, forsitan perennis, supra parce ramosa, ±6 dm. alta, caule subtereti vel moderatim angulato, sulcato. Folia opposita, simplicia, basalia longe tenuissimeque petiolata petiolis usque ad 4.5 cm. longis, laminis oblongo-oblanceolatis vel anguste obtuseque obovatis; principalia caulina sessilia, late oblongo-lanceolata, ciliata, cras-

[12] I have examined 35 collections of *B. ostruthioides* and 14 of *B. costaricensis*.

[13] A form having petioles spiral and tendril-like in their lower ½ or ⅓, these being used by the plant in climbing.

siuscula, apice subacuta, 5–7 (–9.5) cm. longa et 1.2–2 (–3.2) cm. lata. Capitula pedunculata pedunculis 1.5–2.5 dm. longis, radiata, pansa ad anthesin 3.5–4 dm. lata et ±12 mm. alta. Involucri bracteae exteriores 8–10, lanceolatae vel lineari-lanceolatae, tergo glabratae, margine saepe diaphana ciliatae, apice acutae cartilagineaque, 4–7 (rariter –8) mm. longae; interiores late lanceolatae, plerumque 12–14 mm. longae. Flores ligulati circ. 8, flavidi, unicolores, obovati vel late oblanceolati, apice trilobati lobo mediano valde emarginato, circ. 1.5 cm. longi. Paleae superne elongatae et valde caudato-attenuatae. Florum disci stigmata apice caudata. Achaenia suborbicularia, valde obcompressa, dorsaliter convexa, brunneo-atra, alata alis membranaceis planis vel rarissime parce incurvatis 0.2–0.4 mm. latis, apice saepe bidenticulata, faciebus perspicue tuberculata, facie ventrali raro callosa, corpore ipso oblongo-obovato vel late oblongo-oblanceolato 2–3 mm. longa et 1.3–2 mm. lata.

Julian Reverchon 2077, in sandy woods, Mineola, Texas, June 12, 1900 (type in Herb. Berl.: cotype, Herb. Mo. Bot. Gard.); *idem* (*similiter*) 2077, sands, Mineola, Texas, June 10, 1900 (Herb. Mo. Bot. Gard.); *idem* 2041, sands, Big Sandy, Texas, May 27, 1901 (Herb. Mo. Bot. Gard., 2 sheets); *idem*, Pine Island, Texas, May 5, 1903 (Herb. Mo. Bot. Gard.).

The stems of the plants examined are uniformly leafy from bottom to top as in *Coreopsis pubescens* Ell.[14] and the leaves resemble the glabrous undivided ones occasionally found in that species. The elongate peduncles, however, also the pronounced differentiation between exterior and interior involucral bracts, are more as in *C. lanceolata* L.,[15] although not typical for that species.

COREOPSIS MUTICA **leptomera** var. nov.—Glabrata; e specie foliorum glabrorum segmentis tenuiter lanceolatis vel etiam lineari-lanceolatis differt.

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[14] A species of the southeastern United States and ranging as far west as Missouri and Alabama.

[15] A species confined mostly to the eastern United States and southeastern Canada. Specimens have been collected, however, as far west as Wisconsin (*J. H. Schuette*, Door; Herb. Field Mus.), Iowa (*R. Burgess*, Clinton; Herb. Field Mus.), Arkansas (*H. E. Wheeler* 82, near Hazen, Grand Prairie; Herb. Field Mus.), and Texas (*F. Lindheimer*, west of Houston; Herb. Berl.: *W. F. Thurrow*, Hockley; Herb. Univ. Chicago). In China (Provinces of Chekiang, Kiangsi, Honan, etc.) *C. lanceolata* is introduced and now frequent, as shown by various herbarium specimens (Herb. Berl., Herb. Univ. Calif., etc.).

C. G. Pringle 9895, clay banks at altitude of 6800 feet, Dublan, State of Hidalgo, Mexico, Oct. 15, 1902 (type, Herb. Field Mus.: cotype, Herb. Berl.); *C. A. Purpus* 1339, Ixmiguilpan, State of Hidalgo, Mexico, September (Herb. Berl.).

The terminal leaflets are somewhat longer than the lateral and mostly measure 5–7 cm. long and 1.1–1.5 cm. wide. The species proper, which has wider leaf divisions, has been collected in the States of Hidalgo (*Ehrenberg* 354, Herb. Berl.; *C. G. Pringle* 8218, Herb. Berl., Herb. Field Mus.; *idem* 13041, Herb. Berl., Herb. Field Mus.; *C. A. Purpus* 1550, Herb. Field Mus.; *Rose, Painter,* and *Rose* 8837, U. S. Nat. Herb.) and Oaxaca (*C. Conzatti* 2074, Herb. Field Mus.); in Guatemala (*Heyde* and *Lux* 3792, Herb. Field Mus.; *W. A. Kellerman* 6296, Herb. Field Mus.; *H. von Tuerckheim* II. 2043, Herb. Field Mus., 2 sheets), etc. BLAKE (Proc. Amer. Acad. **49**:337. 1913; *cf.* Contr. Gray Herb. new ser. no. **52**:55. 1917) included *Pringle* 9895 under the species itself, but *Pringle* 9895 has foliage of very different aspect from that of DeCandolle's type (Herb. Deless.). This aspect is uniform for both sheets of the *Pringle* material cited, also for all eight of the very leafy branches of *Purpus* 1339 on the sheet cited for the Berlin Herbarium. The absence of intermediate forms, as also the previously known occurrence of two well marked varieties of *C. mutica* (var. *holotricha* Blake and var. *subvillosa* DC.), indicates that we have here another valid variety.

COREOPSIS TRIPTERIS **smithii** var. nov.—E specie foliis omnibus vel prope omnibus integris, lamina tenuiter oblongo-lanceolata differt.

John Donnell Smith, low, open woods near Montgomery, Alabama, Aug. 26, 1885 (type, Herb. Field Mus.); *T. H. Kearney, Jr.*, along Clear Creek, Bell County, Kentucky, September, 1893 (Herb. Field Mus.); *Charles Louis Pollard* 1222, Waynesboro, Mississippi, Aug. 8–9, 1896 (Herb. Field Mus.).

According to *Smith's* additional note on the type sheet, ASA GRAY had pronounced this plant a new variety of *Coreopsis tripteris* L. and, in a letter written in November, 1885, had given it a varietal name. While GRAY would probably have published the name had he not died shortly afterward, I am none the less constrained by the International Rules (Recomm. XIV.*e*) to pass over the name suggested by him and accordingly have named the type after its collector.

Coreopsis scopulorum sp. nov.—Frutex +2 dm. altus, glaber, ramosus, ramis foliosissimis, internodiis saepe tantum 3–5 mm. longis. Folia opposita, petiolata petiolis 6–10 mm. longis, petiolo adjecto tantum 1.2–2 cm. longa, plus minusve biternatisecta, segmentis ultimis linearibus, subcarnosis, margine saepe revolutis, apice mucronatis, vix 1 mm. latis. Capitula tenuiter pedunculata pedunculis 3–5 cm. longis ac saepe ad ramorum terminos 3–5-aggregatis, radiata (radiis in typo deficientibus sed circ. 8 ovariis sterilibus repraesentatis), demum circ. 8 mm. alta et 7–10 mm. lata. Stigmata disci florum apice perspicue caudata. Involucri bracteae exteriores circ. 8, anguste lineares, patenti-reflexae, apice rotundatae vel abrupte cuspidatae, circ. 3 mm. longae, interioribus lanceolatis atque apicem versus reflexis subaequalibus. Paleae perspicuae, late oblongae, apice rotundato-obtusae, achaeniorum corpora moderate superantes. Achaenia linearia, obcompressa, exalata, faciebus marginibusque erecto-setosa, atra, singula facie circ. 8-striata, corpore 5–6 mm. longa et 0.7–1 mm. lata, apice erecto-setosa ac perspicue biaristata aristis tenuibus erecto-hispidis circ. 2.5 mm. longis.

Edmund Heller, on cliffs at altitude of 7100 feet, summit of Mt. Garguez, British East Africa (Kenya Colony), Aug. 26, 1911 (type, U. S. Nat. Herb., no. 634308).

Apparently nearest *Coreopsis elgonensis* Sherff, from which it differs in its longer and petiolate leaves, its shorter external involucral bracts, its aristate achenes, etc.; and *C. chippii* Moss,[16] from which it differs in its much broader leaf-segments, etc. All three species offer a stronger resemblance to certain of the low, shrubby South American species of *Coreopsis* than to the various African species.

COREOPSIS JACKSONI **arthrochaeta** var. nov.—A specie foliis maximam partem dense hispidis, setis albidis elongatis sensim attenuatis multiloculatis differt.

R. L. Piemeisel and *L. W. Kephart* 166, at altitude of 3300 m., vicinity of Camp Gusisu, Aberdares Mts., British East Africa (Kenya Colony), Jul. 29, 1927.

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[16] I am indebted to DR. ARTHUR W. HILL, Director of The Royal Botanical Garden of Kew, for his having sent me a photograph and type fragment of the very recently described *C. chippii*.

The species proper[17] is known to me through the type, collected by *Frederick J. Jackson*, Kikuyu region, British East Africa, 1889 (Herb. Brit. Mus.), also through *Dr. Edgar A. Mearns* 1291 and 1722, Mt. Kenia, British East Africa, September–October, 1909 (U. S. Nat. Herb.). The leaves are essentially glabrous. The material collected by *Piemeisel* and *Kephart* consisted of at least six small, entire plants and these offer a strikingly unique appearance because of their densely hispid leaves. Since endemism is pronounced among the plants of the Kikuyu region, and since elsewhere even where endemism is relatively unimportant (the United States) *Coreopsis* displays several forms that are accepted as varieties, it appears worth while to distinguish the *Piemeisel* and *Kephart* plants as connoting a group of varietal rank.

COREOPSIS TRIPTERIS **subrhomboidea** var. nov.—Folia tripartita foliolis lateralibus lanceolatis terminali rhomboideo-lanceolato 1.7–2.3 cm. lato, petiolo adjecto 6–8 cm. longa. Capitula minora, achaeniis tantum 4–4.5 mm. longis.

Ernest Jesse Palmer 29421, sandy, open woods bordering bog, near Texarkana, Bowie County, Texas, Oct. 27, 1925 (type, Herb. Gray).

In the more than two hundred collections of the species proper studied by me, all the leaflets were variously linear, oblong-linear or narrowly oblong-lanceolate, and the achenes were commonly 5–6 mm. long. In the *Palmer* plant the terminal leaflets approach very distinctly a rhombus in outline and the achenes are smaller, measuring only about 4–4.5 mm. in length.

COSMOS CARVIFOLIUS Benth. Bot. Voy. Sulphur 117. 1844; *Bidens carvifolia* (Benth.) Schz. Bip. *in* Seem. Bot. Voy. Herald 308. 1852–1857; *B. seemannii* Schz. Bip. *in* Seem. *loc. cit.* 307; *Cosmos*

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[17] Formerly I had assumed (*cf.* BOT. GAZ. 81:45. 1926) that the species was best interpreted as belonging in *Bidens*. The recent finding, however, of a mature specimen with numerous ripe achenes (*Mearns* 1722, U. S. Nat. Herb. no. 631676) showed that there had been no warrant for transferring the species to *Bidens*. The mature achenes of the species proper are seen to be blackish or brownish-black, thick-clavate, more or less swollen and quadrangulate, not visibly striate, about 3.2–3.7 mm. long and 1.1–1.7 mm. thick, glabrate, exalate and exaristate but at the apex often accompanied by a basal remnant of the withered disc floret (giving it the appearance of having a short thick beak). This last character is unknown to me elsewhere either in *Bidens* or in *Coreopsis*, although of course in certain related genera (e.g. *Heterosperma*) some achenes have a true beak or neck looking somewhat like the disc floret's basal remnant here.

seemannii (Schz. Bip.) Gray, Proc. Amer. Acad. **19**:16. 1883; *etiam ex* Greenm., *ibid.* 41:265. 1905; *Bidens seemanii* Schz. Bip. *ex* Sherff, Bot. Gaz. **64**:28. 1917 (sphalm).—The type of this species was collected by *Mr. George Barclay*, of the Sulphur Expedition, at Tepic, Territory of Tepic, Mexico. In herbaria, however, the various additional specimens collected later in the same and other regions of Mexico have been referred quite uniformly to *C. seemannii* (Schz. Bip.) Gray. The types of both *C. carvifolius* and *C. seemannii* are still preserved at Kew and are seen to be identical. The former, however, is of a plant that has a somewhat irregular growth habit, with more than the usual number of branches and the more numerous heads rather stunted in size. Bentham compared his type with *C. bipinnatus* Cav., a species having 2- (rarely 3-) aristate achenes, while his type had 5-aristate achenes. More commonly the achenes are 6–8-aristate.[18]

Specimens examined: *Brother Arsène*, Cerro San Miguel, Morelia, State of Michoacan, February, 1909 (Herb. Deless.; Herb. Field Mus.; Herb. Phila.); *George Barclay* (Voyage of the Sulphur), Tepic, Territory of Tepic, 1836–1842 (type, Herb. Kew); *Dr. Ghiesbreght* 264, cold ground, September-October (Herb. Gray, 2 sheets); *Ynes Mexia* 601, common at alt. 1000 m., on open hillside, road from Tepic to Jalcojotan, State of Nayarit, Sept. 15, 1926 (U. S. Nat. Herb.); *Dr. Edward Palmer* 1852, Tepic, Territory of Tepic, Jan. 5–Feb. 6, 1892 (Herb. Field Mus.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *C. G. Pringle* 8845, alt. 5000 ft., in fields, Uruapan, State of Michoacan, Oct. 8, 1904 (Herb. Carnegie Mus.; Herb. Deless.; Herb. Gray; Herb. Phila.; Herb. U. S. Nat.); *Joseph N. Rose* 3435, in the Sierra Madre, near Santa Teresa, Territory of Tepic, Aug. 11, 1897 (U. S. Nat. Herb.); *Berthold Seemann* 2014 (Voy. of the Herald), Sierra Madre, Mexico (Herb. Gray; Herb. Kew; type collection of *Cosmos seemannii*).

Cosmos gracilis sp. nov.—Herba annua, erecta, glabra, gracilis, infra simplex supra ramosa, 4–6 dm. alta, caule subtetragono, internodiis quam foliis multo brevioribus. Folia tenuiter petiolata petiolis parce marginatis usque ad 1.5 cm. longis, petiolo adjecto 4–6 cm. longa, bipinnatisecta, segmentis membranaceis saepius oblongo-linearibus, margine subciliatis, apice acutis, plerumque 1–3 mm. latis. Capitula tenuiter pedunculata pedunculis usque ad 16 cm. longis, non numerosa (unicae plantae circ. 5–12), radiata, pansa ad

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[18] *Palmer* 1852, from the type locality of Tepic, has the achenes mostly 5-aristate as in Bentham's type. These are accompanied however by a few achenes with 6 aristae and several with only 4.

anthesin circ. 1.8 cm. lata et circ. 1 cm. alta. Involucri glabri bracteae exteriores 5–7, anguste cuneato-lanceolatae, obsolete nervatae, saepe patentes, tantum 1.5–2.2 (raro –4) mm. longae; interiores oblongo-lanceolatae demum circ. 7–8 mm. longae. Flores ligulati circ. 5 vel 6, flavidi (non aurantiaci), ligula obovati, apice truncato-lobulati, tantum circ. 6–8 mm. longi. Paleae lineari-lanceolatae. Achaenia circ. 6–10, fusiformia, rotundato-tetragona, unaquaque facierum 1-sulcata, infra nigra glabrataque supra rostrato-angustata ac straminea erecto-hispidulaque, apice spinulis patentibus minutissimisque coronata vel demum calva, 1.1–1.7 cm. longa et circ. 1.1–1.3 mm. crassa.

Henri François Pittier 5053, at altitude of 100–350 meters, vicinity of Olá, Province of Coclé, Panama, Dec. 7–9, 1911 (type, U. S. Nat. Herb.: cotype, Gray Herb.).

Differs from *Cosmos sulphureus* Cav. in having smaller size, more delicate habit, the stem glabrous with the internodes shorter than the leaves (these smaller and less compound), the flowering and fruiting heads much smaller, the exterior involucral bracts proportionately smaller, the rays golden yellow not orange, etc.

COSMOS DIVERSIFOLIUS **pumilus** var. nov.—Herba pumila, 3–4 dm. alta, foliis basalibus, pedunculis scaposis, capitulis pansis ad anthesin tantum circ. 4 cm. latis, radiis subalbidis et parce violaceis, achaeniis atris erostratis corpore tantum 6–10 mm. longis et 0.7–1 mm. crassis, apice plerumque 2- (raro usque ad 4-) aristatis aristis tantum circ. 1 -1.5 mm. longis.

Carl Albert Purpus 3029, in meadows, Boca del Monte, State of Puebla, Mexico, September, 1908 (type, Herb. Field Mus.: cotypes, Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

COSMOS OCELLATUS **greenmanii** var. nov.—A specie ipsa foliis plerumque tripartitis foliolis ovato-lanceolatis serratisque, achaeniis etiam juvenibus apice exaristatis differt.

Cyrus Guernsey Pringle 8386 *pro parte*, at altitude of 7500 feet, in thin soil on the knobs of the Sierra de Tepoxtlan, State of Guerrero, Mexico, Oct. 14, 1900 (type, Herb. Field Mus.: cotypes, Herb. Mo. Bot. Gard., etc.).

Named in tribute to DR. JESSE MORE GREENMAN, Curator of the Herbarium of the Missouri Botanical Garden (*cf.* footnote 9). The species proper, originally described by GREENMAN from other speci-

mens by *Pringle* (under the same number), has usually 2–3-pinnate leaves, achenes 2-aristate, etc.

COSMOS PEUCEDANIFOLIUS Wedd. Chlor. And. 1:70. 1855; *Bidens peucedanifolius* (Wedd.) O. Ktze. and vars. *bipinnatisecta* O. Ktze. and *soratensis* O. Ktze., Rev. Gen. 3^{II}:137. 1898.—In the past, several students of South American Compositae have regarded *Cosmos diversifolius* Otto, a species originally described from Mexican material, as extending from Mexico into Peru and Bolivia. A comparative study of many specimens from northwestern South America and from Mexico shows, however, that the former, while differing among themselves considerably, are fairly well marked and can usually be distinguished from *C. diversifolius* without much difficulty.

WEDDELL (*loc. cit.*) was the first to segregate the South American material. He described three species—*C. integrifolius*, *C. peucedanifolius*, and *C. subpubescens*. The first and last of these are treated in the immediately following sections of this article. His *C. peucedanifolius*, from Bolivia, may best be recognized by the narrowly linear leaf segments: "foliis profunde pinnatisectis, lobis utrinque 2 anguste linearibus elongatis integris, terminali caeteris fere duplo longiore. . . ." (Wedd. *loc. cit.*). OTTO KUNTZE's vars. *bipinnatisecta* and *soratensis* are represented by good specimens still extant (Herb. Berl.; Herb. N. Y. Bot. Gard., etc.) and are seen to belong here, differing only in the here unimportant matter of degree of leaf dissection.

Specimens examined: *Miguel Bang* 1302, vicinity of Sorata, Bolivia, May 1892 (Herb. Gray; Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Herb. Phila.; Herb. U. S.Nat.; Herb. Univ. Vienna; type collection of *Bidens peucedanifolia* var. *soratensis* O. Ktze.); *Dr. Otto Buchtien* 611, alt. 3200 m., mountain slopes, Unduavi, North Yungas, Bolivia, February, 1915 (Herb. Field Mus.; Herb. U. S. Nat.); *Cardenas* "7 special," Cochabamba, Bolivia, 1922 (Herb. U. S. Nat.); *K. Fiebrig* 2966, alt. 3300 m., Patanca, southern Bolivia, Feb. 1, 1904 (Herb. Gray); *Dr. Otto Kuntze*, alt. 3000 m., Rio Juntas, Bolivia, April, 1892 (Herb. N. Y. Bot. Gard.; cited by *Kuntze* for his *Bidens peucedanifolia* var. *bipinnatisecta); idem*, alt. 3400 m., Tunari, Bolivia, May 4, 1892 (Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Kuntze's first cited specimen of his var. *bipinnatisecta); Dr. H. H. Rusby* 1682 *pro parte*, alt. 10000 ft., Sorata, Bolivia, February, 1886 (Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.); *C. H. T. Townsend* 1507, Pachicayo, Peru, Mar. 27, 1913 (Herb. U. S. Nat.).

COSMOS PEUCEDANIFOLIUS var. **cochabambensis** (O. Ktze.) comb. nov.; *Bidens peucedanifolia* var. *cochabambensis* O. Ktze. Rev. Gen. 3 ^{II}:137. 1898; *Cosmos integrifolius* Wedd. Chlor. And. **1**:70. 1855 (*ex descript. et patr.*).—*C. integrifolius* apparently was segregated from *C. peucedanifolius* mainly because of its leaf characters: "foliis lineari-lanceolatis, decimetralibus, integerrimis rariusve lobo lineari ad unum alterumve latus vel utrinque instructis. . . ." (Wedd. *loc. cit.*). The type collection of KUNTZE's var. *cochabambensis* (*Bang* 1021) shows a slight tendency toward greater division of the leaves, but otherwise agrees with WEDDELL's description of *C. integrifolius*. KUNTZE's interpretation of this form, as representing a variety, is doubtless nearer the truth than was WEDDELL's.

Specimens examined: *Miguel Bang* 1021, vicinity of Cochabamba, Bolivia, 1891 (Herb. Gray; Herb. Mo. Bot. Gard.; Herb. Phila.; Herb. U. S. Nat.; type collection).

COSMOS PEUCEDANIFOLIUS var. **tiraquensis** (O. Ktze.) comb. nov.; *C. subpubescens* Wedd. Chlor. And. **1**:70. 1855 (*ex descript. et loco*); *C. pulcherrimus* Schz. Bip., Bull. Soc. Bot. Fr. **12**:79. 1865 (*nomen subnudum*); *Bidens pulcherrima* Schz. Bip., Linnaea **36**:528. 1865–1866 (*nomen subnudum*); *Cosmos marginatus* Klatt, Abhandl. Naturf. Ges. Halle **15**:328. 1882; *Bidens peucedanifolia* var. *tiraquensis* O. Ktze. Rev. Gen. 3 ^{II}:137. 1898.—WEDDELL's *C. subpubescens* was based on a plant by *Gay* from the Province of Cuzco, Peru. A careful reading of his description shows that here again the leaves were the chief basis of distinction: "foliis pinnatisectis, lobis utrinque 2–3-lanceolatis integris vel pauci-dentatis mucronatis. . . . ." There have come from Cuzco and the adjacent region many specimens which match WEDDELL's description fairly well. One collection was by *A. Mathews* from the Province of Chachapoyas, Peru. This was the basis of *Cosmos marginatus* Klatt. Two others, by *G. Mandon* and both under his no. 54, were from the vicinity of Sorata, Bolivia, and were the basis of the names (*sine descript.*) *Cosmos pulcherrimus* Schz. Bip. and *Bidens pulcherrima* Schz. Bip. Another was by *Dr. Otto Kuntze* from Tiraqui, Bolivia. This was the basis of *Bidens peucedanifolia* var. *tiraquensis* O. Ktze. It is through this form, which cannot be regarded as of higher than varietal rank, that

C. peucedanifolius makes its nearest approach to low and rather dwarfed forms of *C. diversifolius* Otto.[19]

Specimens examined: *Mrs. Adolph F. Bandelier* 18, alt. 12500 ft., Isl. Titicaca, Lake Titicaca, Bolivia, 1905 (Herb. N. Y. Bot. Gard.; *nomen incolarum aymaranarum* Panti-Panti); *Dr. Otto Buchtien* 3074, alt. 3840 m., Isl. del Sol, Lake Titicaca, Bolivia, March, 1910 (Herb. Field Mus.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *K. Fiebrig* 2822, alt. 3000 m., Pinos near Tarija, southern Bolivia, Jan. 22, 1904 (Herb. Deless.; Herb. Field Mus.; Herb. Mo. Bot. Gard.); *F. L. Herrera*, alt. 3000–3600 m., Cuzco, Peru, July, 1923 (Herb. U. S. Nat.); *idem* 1025, alt. 3700 m., Hacienda Churu, Province of Paucartambo, Peru, January (*e pittacio lectoris ipsius*), 1926 (Herb. Field Mus.; Herb. Gray, 2 sheets; Herb. Mo. Bot. Gard.; Herb. U. S. Nat.; *nom. incolarum* Panti); *Dr. Otto Kuntze*, alt. 4000 m., Tiraqui, Bolivia, Apr. 1–4, 1892 (type, Herb. N. Y. Bot. Gard.); *G. Mandon* 54, alt. 2650–3000 m., in uncultivated places, thickets, etc., vicinity of Sorata, along road to Lucatia, Bolivia, February–March, 1858 (Herb. Deless., 3 sheets; Herb. N. Y. Bot. Gard.); *idem* (*similiter*) 54, alt. 2800–3000 m., in thickets everywhere, vicinity of Sorata, January–March, 1859 (Herb. Deless.; Herb. Gray; Herb. N. Y. Bot. Gard.); *Alexander Mathews*, Province of Chachapoyas, Peru (Herb. Gray, 2 sheets; type material of *C. marginatus* Klatt); *Dr. H. H. Rusby* 1682 *pro parte*, alt. 10000 ft., Sorata, Bolivia, February, 1886 (Herb. Gray; Herb. N. Y. Bot. Gard., *cum spec. ipsa;* Herb. Phila.; Herb. U. S. Nat.); *A. Weberbauer* 7597, alt. 3600 m., on grass steppe with scattering shrubs, Yanahuajra Valley, Department of Ayacucho, Province of Huanta, Peru, Mar. 18, 1926 (Field).

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

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EXPLANATION OF PLATES XVII–XXI

PLATE XVII

Bidens pilosa var. *bimucronata* f. *odorata* (*B. inermis* Wats.): *a*, flowering and fruiting branch, ×0.65; *b*, lower cauline leaf, ×0.65; *c*, portion of stem magnified to show details, ×2; *d*, exterior involucral bract, ×4; *e*, interior involucral bract, ×4; *f*, ray floret, ×4; *g*, palea, ×4; *h*, disc floret, ×4; *i*, anthers, ×20; *j*, style branches, ×20; *k*, *l*, achenes, ×4.6; *m*, pollen grain, ×385; all from *C. G. Pringle* 1291, Herb. Field Mus.

PLATE XVIII

Bidens coriacea: *a*, flowering and fruiting branch, ×0.62; *b*, exterior involucral bract, ×2.6; *c*, *d*, *e*, various interior involucral bracts, *c*, *e*, ×2.6, *d*,

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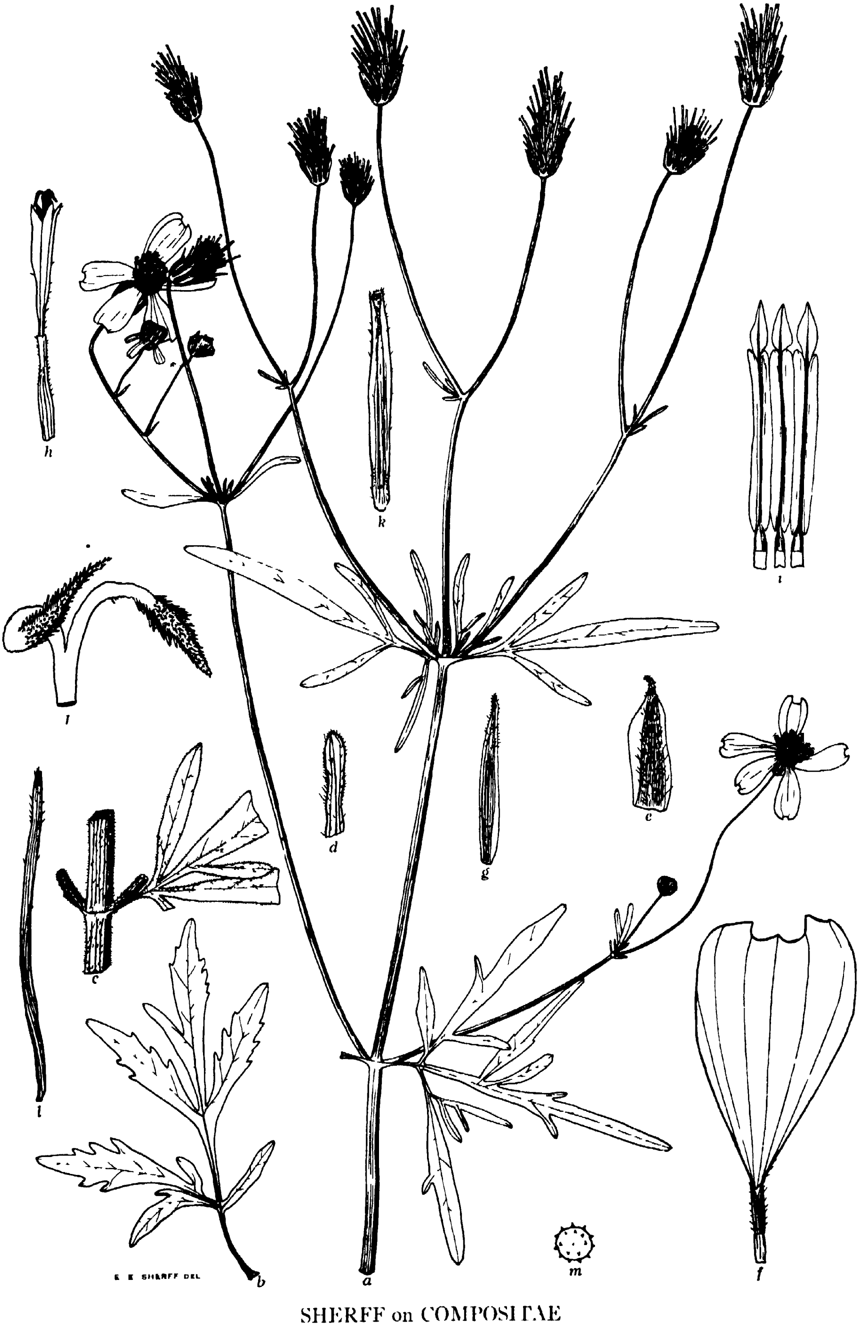
[19] My present treatment is necessarily abridged. Further details are reserved for my forthcoming *Revision of the genus Cosmos.*



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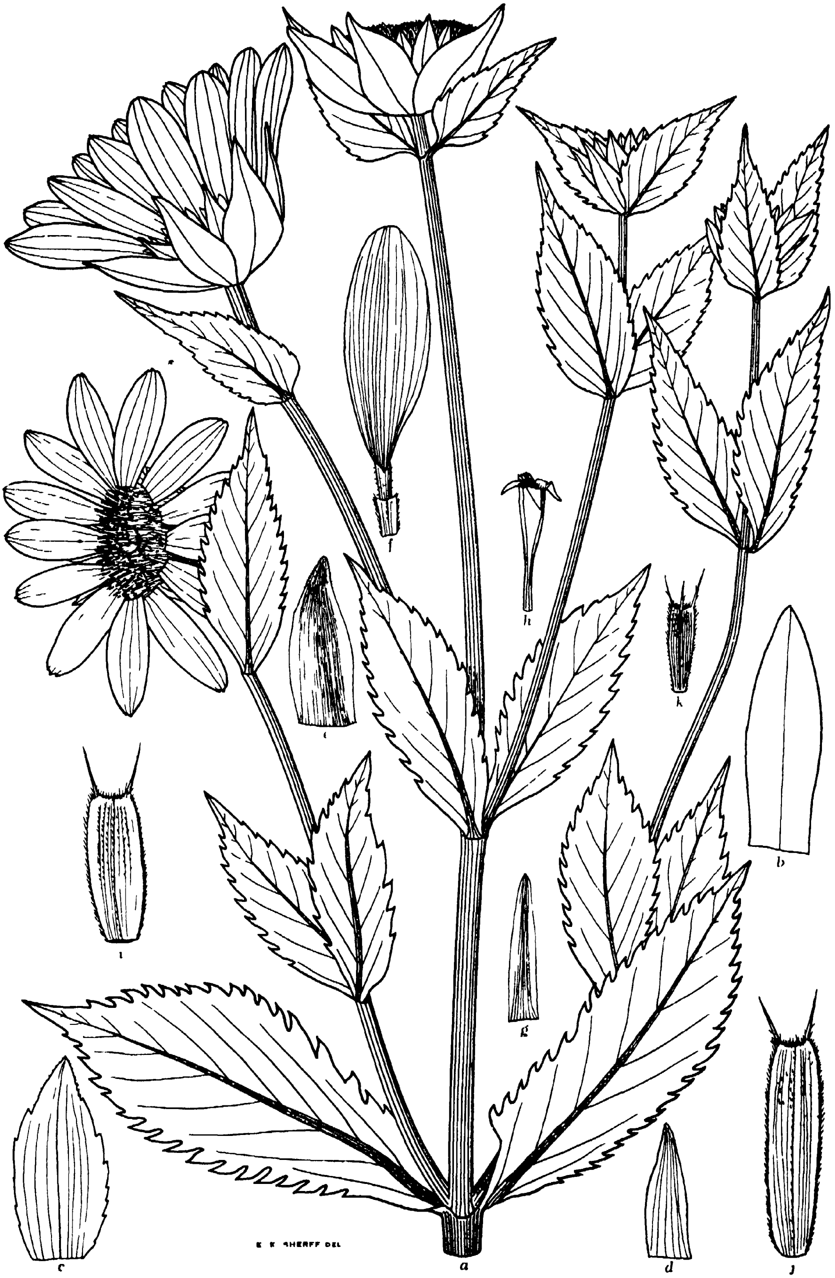

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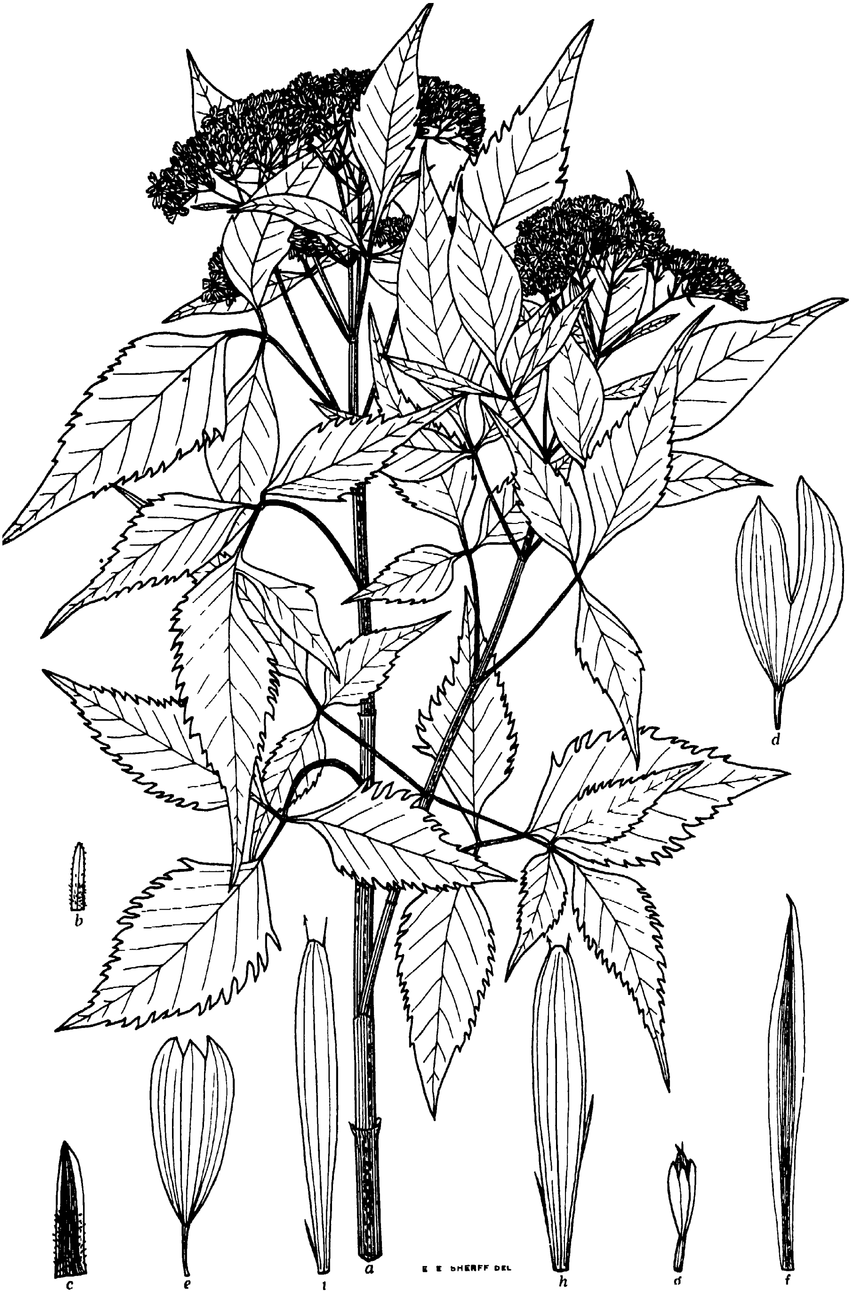

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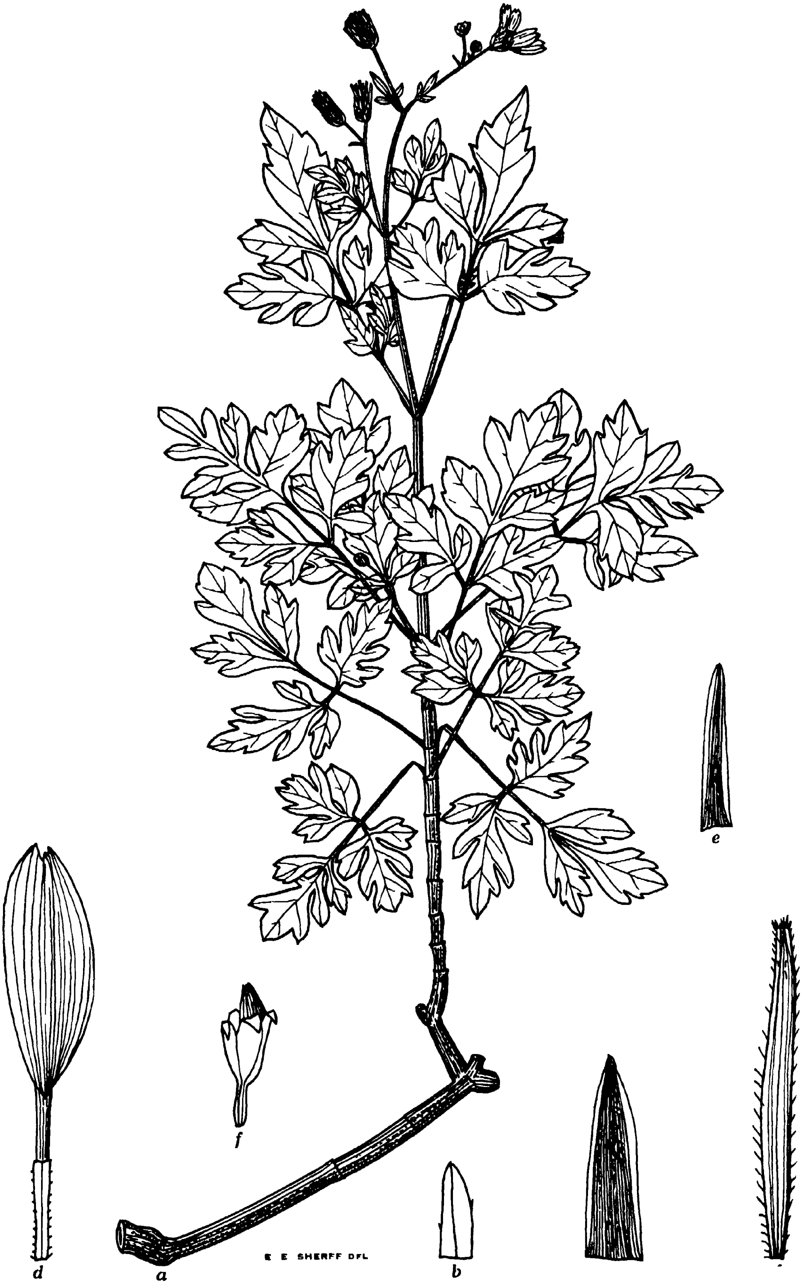

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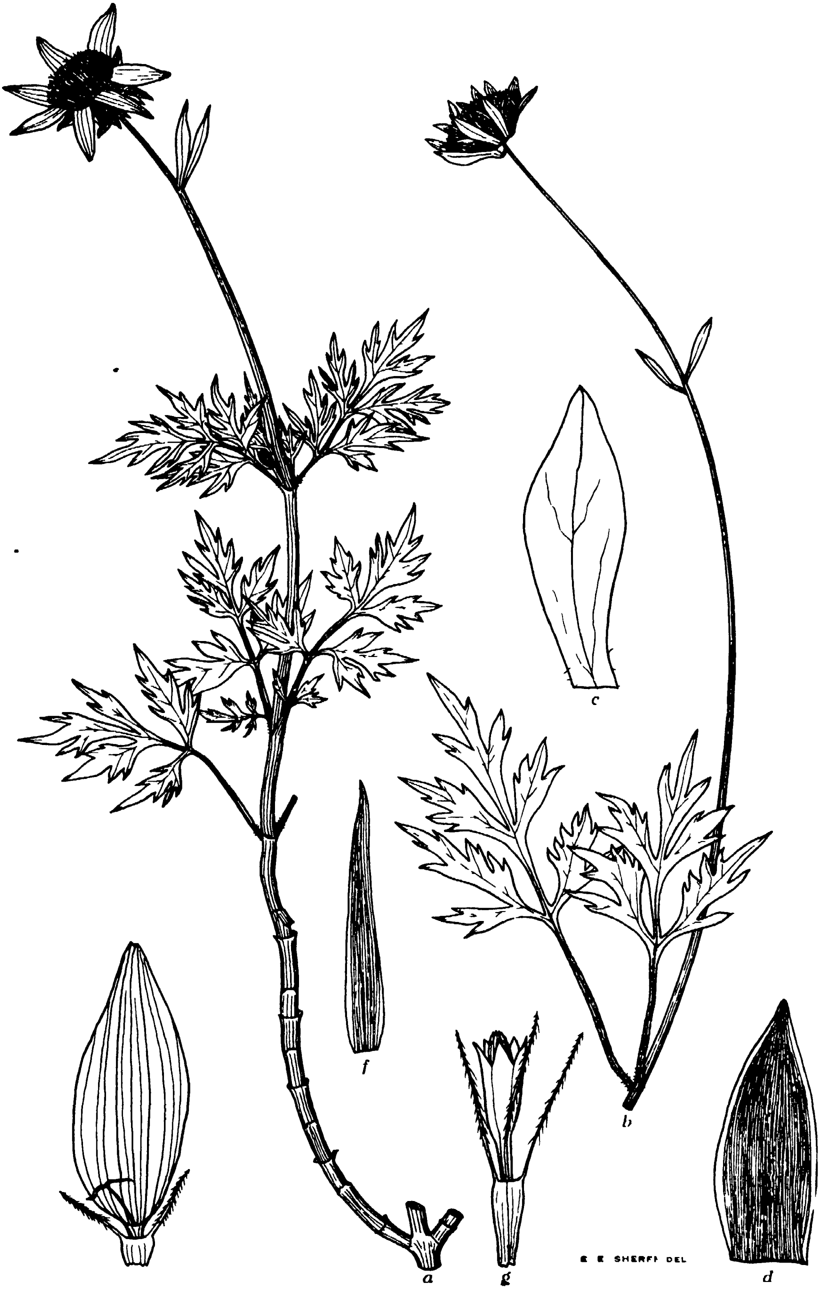

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×1.9; *f*, ray floret, ×1.9; *g*, palea, ×1.9; *h*, disc floret, ×3.9; *i*, *j*, achenes, ×3.9; *k*, achene, ×1.9; *a*, from *Mr. J. D. Snowden* 28, alt. 4700 ft., Uganda, British East Africa, 1913, Herb. Kew; *b*, *d*, *f*, *g*, *h*, *i*, *j*, from *G. F. Scott Elliot* 7410, cotype of *Bidens ruwenzoriensis* (S. L. Moore) Sherff in Herb. Kew; *c*, *e*, *k*, from *Fischer* 367, type of *B. coriacea* in Herb. Berl.

PLATE XIX

Bidens schizoglossa: *a*, flowering and fruiting branch, ×0.68; *b*, exterior involucral bract, ×6.8; *c*, interior involucral bract, ×6.8; *d*, *e*, rays, ×6.8; *f*, palea, ×6.8; *g*, disc floret, ×6.8; *h*, *i*, achenes, ×6.8; all from *W. A.* and *C. B. Setchell*, near Huehue, Isl. Hawaii, June 24, 1924, type in Herb. Univ. Calif.

PLATE XX

Bidens obtusiloba: *a*, flowering and fruiting branch, ×0.74; *b*, exterior involucral bract, ×7.4; *c*, interior involucral bract, ×7.4; *d*, ray floret, ×7.4; *e*, palea, ×7.4; *f*, disc floret, ×7.4; *g*, achene, ×7.4; all from *D. LeRoy Topping* 2941, type in Herb. Univ. Calif.

PLATE XXI

Bidens ostruthioides var. *costaricensis:* *a*, *b*, flowering branches, ×0.68; *c*, exterior involucral bract, ×4; *d*, interior involucral bract, ×4; *e*, ray floret, ×2.7; *f*, palea, ×4; *g*, disc floret, ×4, all from sheet of assorted *Oersted* materials from Mt. Irazú, Mt. Aguacate, etc., in BENTHAM's herbarium in Herb. Kew.

PHYSIOLOGICAL IMPORTANCE OF CALCIUM IN LEGUME INOCULATION

W. A. ALBRECHT AND F. L. DAVIS

(WITH FOUR FIGURES)

Introduction

That calcium should be an important factor in the nodulation of legumes on acid, or sour, soils is suggested by the varying results in nodulation obtained on these soils. This is indicated especially in the soils of northeastern Missouri and southern Illinois, where the many or even frequent and repeated failures of inoculation with pure cultures have been found to occur on certain predominating acid soil types; while improved inoculation results when these soils are supplied, either previous to or at the time of planting, with limestone, acid phosphate, or other calcium-bearing materials.

Data presented by HELLRIEGEL and WILFARTH, in their original article (9), establishing the relation between legume root nodules and nitrogen fixation, show a stimulating effect of calcium upon nodulation and growth of serradella. Recently ALWAY (1), in comparing the effectiveness of soil transfer with that of pure cultures as inoculation for alfalfa on lime-deficient, sandy soils, found that when the land had not been limed the soil transfer method was far more effective for the first seeding. Excessive increases in the amount of culture did not make this method as effective as soil transfer. On land limed well in advance of seeding, however, the inoculations by soil transfer and by the pure culture method were of equal efficacy. This seems to indicate an inability of the organisms to establish themselves quickly in a lime-deficient soil habitat.

FELLERS (8), in a summary of his work on the factors affecting nodulation of soy beans, states: "The bacterial infection of roots does not take place readily on acid soils even when the root infecting organisms are plentiful in the soil." BRYAN (3), in a study of the effect of acid soil reactions on nodulation of soy beans, found that in general the hydrogen-ion relations for the organism tend to be the same as those for the host plant. He secured a maximum nodulation at pH 6.5, and none below pH 4.9, although the critical hydrogen-ion concentration for the organisms in solution cultures was found to be pH 3.5–3.9.

TRUOG (15) ventured the theory that the effect of soil reaction on the activity of the bacteria within the nodule is indirect, since the environment of the bacteria when in the nodule must be that of the plant tissue. KARRAKER (11), in examining this viewpoint, found that the root system of a single alfalfa plant, divided between a lime-deficient and a limed soil, gave differences in nodule formation corresponding to those obtained on different plants growing wholly within these different soils. He concluded that the effect of soil reaction on nodule formation must be one of localized character in the plant, a direct effect of soil pH on the bacteria in the nodules, or an antecedent effect of the soil acidity on the bacteria, while they are existing non-symbiotically in the soil.

SCANLAN (14) concluded that hydrogen-ion concentration has no direct effect upon inoculation, and analogous inoculation resulted from the use of limestone and calcium acetate where the former lessened the hydrogen-ion concentration while the latter had no effect upon it.

FALK (7) thinks that neutralization of the acid is not the only effect of certain valuable salts, this being indicated by the fact that magnesium carbonate and phosphate improve bacterial growth more effectively than calcium carbonate. MACHIDA (12) demonstrated that calcium salts and not magnesium salts are effective in protecting bacteria against lethal agencies. This work was substantiated by CHAMBERS and REZINKOFF (5), studying the protoplasm of *Amoeba proteus*.

WINSLOW and FALK (16) found that concentrations of 0.4 per cent of sodium chloride alone, or of 0.2 per cent of calcium chloride alone, exerted marked lethal effects upon the growth of a typical colon bacillus, *B. communis*, while, a mixture of these two, in the ratio of one Ca-ion to five Na-ions and in concentrations as high as 5 per cent of the salt, was actually beneficial in its effect upon the growth of the organism. HOTCHKISS (10) made a survey of the effects of cations upon the bacterial growth of *B. coli*, and found that calcium chloride in a 0.5 molar concentration limited growth completely, but stimulated growth in a 0.05 molar concentration. SCANLAN (14) found that one part of calcium chloride to 1500 parts of solution was the optimum concentration for stimulating growth and longevity of *B. radicicola.*

Investigations to date give numerous observations on the effects of limed and acid soils on nodulation of legumes, with almost as many theories as to the operating causes. These conditions emphasize the need for a fuller understanding of the fundamental facts controlling the responses in nodule production by legumes and their bacteria on soils of varying degrees of soil acidity or base deficiency.

Experimentation

PART I

The work here reported bears testimony to some of the preceding viewpoints. Results analogous to those of KARRAKER were obtained in working with soy beans on an acid Putnam silt loam. Seedlings were grown in sterile sand for 10-14 days, when the tap roots were cut off just below the lateral roots, which had developed in good numbers and to a length of about 1 inch. These seedlings were placed over a water-tight partition in a container with one-half of the root system carefully planted into an acid soil (pH 5.14) on one side, and the other half into the same soil after it had been thoroughly mixed with calcium carbonate at the rate of 8000 pounds per two million of soil. Liberal quantities of a suspension of inoculating bacteria were added as the soil was filled into the pans around the root system. Five seedlings with their lateral roots so divided were set into each container. In addition, ten seedlings, with their tap roots likewise cut off, were planted into the container, five on the side of the acid soil and five on the side of the limed soil. Water was maintained at the optimum by daily surface applications.

Although there was a high mortality of plants in consequence of tap root pruning and replanting, those that lived grew very satisfactorily. At the end of 5 weeks they were carefully taken up and the nodules counted. The nodule counts of the plants with divided roots and checks are summarized in table I. The results show an increase of 208 per cent in the number of nodules formed on the portion of the roots growing in the calcium soil as compared with those formed on the portion in the untreated soil. The difference of 181.1 per cent in nodulation by the check plants, grown wholly within one kind of soil, is approximately the same ratio. The comparison of the degrees of inoculation of the divided root plants and the checks is made on this basis rather than on the actual plant units, because the average nodulation of either portion of the root of the divided root plants represents but one-half of the normal nodulation of the plants.

TABLE I

NODULATION OF PLANTS GROWN WITH PART OF ROOT SYSTEM IN CALCIUM-TREATED SOIL AND PART IN UNTREATED SOIL (pH 5.14)

TREATMENT	PLANTS WITH DIVIDED ROOTS			CHECK PLANTS			
		Nodule production		Calcium-treated soil		Untreated soil	
	No. of plants	Roots in calcium-treated soil	Roots in untreated soil	No. of plants	No. of nodules	No. of plants	No. of nodules
Uninoculated	4	0	0	6	0	5	0
Inoculated	23	160	77	28	494	31	302
Average per plant	......	6.95	3.34	....	17.64	......	9.74
Percentage increase	......	208.0	....	....	181.1	.....	....

The reliability of these data is questionable on account of, first, the small number of plants on which the test was completed, and second, the unequal development of the two parts of the divided root systems.

The results obtained for soy beans agree well with those for alfalfa by KARRAKER (11). They indicate that, if the effect of the calcium is a physiological one through the plant, this effect is local in character, and the calcium is certainly not translocated to all the roots of the plant for equal effectiveness in improving inoculation; or, that the depressed nodulation in this acid soil is due to an effect of the soil conditions upon the bacteria before they have infected the roots of the host plant.

PART II

A test of the stimulating effect of calcium upon nodulation of soy beans on an already well inoculated soil emphasized further the possible physiological effect of this element. The soil used had a pH of 5.5, and was well stocked with the soy bean organism in consequence of well inoculated crops of soy beans on three previous seasons.

Enough of this soil was mixed with calcium carbonate at the rate of 8000 pounds per two million to fill thirty pots, while a like number were filled with untreated soil, and each of the sixty pots was planted with five sterile, sprouted soy beans. After cautious culture for five weeks, when the plants showed a uniform healthy growth with slight superiority in color, size, and appearance in the case of those given calcium carbonate, counts of the nodules were made as given in table II. The results show an increase of 336 per cent in the numbers of nodules as a result of liming, in spite of the fact that the soil was already well inoculated with the organisms. This agrees with FELLERS' observation, and while it demonstrates the correlation of nodulation of legumes on acid soils with the acidity, it does not establish the relation of cause and effect between them.

TABLE II

NODULE NUMBERS AS INFLUENCED BY CALCIUM CARBONATE ON SOIL GROWING SOY BEANS PREVIOUSLY*

SOIL TREATMENT	POTS	PLANTS	NODULE NUMBERS				
			Total	Range per pot	Average per pot	Average per plant	Increase per cent
None	30	130	1659	10–142	55.3	12.0	
Calcium carbonate	30	133	5353	64–380	178.4	40.2	336.8

* Silt loam soil pH 5.5.

PART III

In order to differentiate more closely between the effect of soil reaction upon nodulation and the importance of calcium to nodulation, a study of the effect of calcium, as calcium chloride, on the viability of *B. radicicola* was made. For this a rather well isolated soil, mainly of residual formation from limestone, was collected from a timbered area. It was decidedly acid (pH 5.4), and sterile with reference to the soy bean organism. Sixty pots (3.5 inches in diameter) of this soil were set up, planted to soy beans, and given thorough inoculation with cultures of bacteria. One-half of these pots were given the additional treatment of a solution supplying 88 mg. of calcium chloride per pot, the calcium equivalent of 2000 pounds of calcium carbonate per two million of soil. Eight additional pots without inoculation or calcium treatment were used as checks. The nodule counts were made after growth of five weeks.

Not a nodule had formed on a single plant in the untreated soils, and but three single nodules on as many plants in the calcium-treated soil, although a good growth of plants was obtained in all the pots. In order to test the soil for the presence of the organism, sixteen pots of each of the treated and untreated soil were immediately replanted with sprouted beans. No further inoculation or treatment was added. Again after five weeks of growth these were taken up and the nodules counted as before. The data are summarized in table III.

TABLE III

NODULE NUMBERS OF SOY BEANS AS INFLUENCED BY CALCIUM CHLORIDE TREATMENT ON SOIL

	TREATMENT	POTS	PLANTS	STERILE PLANTS	INOCULATED PLANTS	TOTAL NODULES
Inoculated first planting	None......	30	130	130	0	0
	Calcium chloride...	30	133	130	3	3
Uninoculated second planting	None.......	16	72	69	3	3
	Calcium chloride.	16	80	0	80	146

The results of this trial emphasized the difficulty of establishing the soy bean organism within this soil by a single inoculation, and demonstrated that even though the organism did not exist in the untreated soil, it continued its existence in the calcium-treated soil despite no significant change in the soil reaction. This indicates, in substantiation of SCANLAN'S findings, that the response by the organism must be due to effects by the calcium.

PART IV

Since calcium seemed to favor nodulation, an attempt was made to determine whether or not this influence comes in consequence of its direct effect on the soil conditions, or of an indirect effect through the plant. Quartz sand was treated with hydrochloric acid for 3 hours, washed with tap water and then with distilled water until the test for chlorides was negative. The sand was dried, and heated at 111° C. for 48 hours for drying and for sterilization. Part of the sand was treated with calcium carbonate at the rate of ten thousand pounds per two million pounds of sand.

Sterilized soy beans, the progeny from a single plant, were germinated for 24 hours, planted in both the limed and unlimed sand, and then grown for 10 days. Seedlings from both the calcium-bearing and the calcium-deficient sands were washed free of adhering particles and transplanted into the respective halves of each of two flats of well prepared soils. One of these soils was a lime-deficient field soil and the other a neutral garden soil, both of which had grown well-inoculated soy beans during the three preceding seasons. Excellent growth resulted in both flats, and after 5 weeks the plants were removed and counts made of the nodules, with the results given in table IV.

TABLE IV

NODULE PRODUCTION BY SOY BEANS IN NEUTRAL AND ACID SOILS AS INFLUENCED BY CALCIUM IN TRANSPLANTED SEEDLINGS

KIND OF SOIL	PH	SEEDLING TREATMENT	NO OF PLANTS	NODULE NUMBERS		CALCIUM CONTENT (MG CA)		
				Range per plant	Average per plant	Seedlings	Soil	Seeds
						Per 100	Electrodialysable per 10 gm W-free	Per 100
Neutral	7 8	None	60	12–77	36 6	17 07	24 07	6 85
		Calcium	67	9–67	38 9	30 14		
Acid	5 5	None	69	1–7	3 4	17 07	11 78	6 85
		Calcium	79	2–25	15 1	30 14		

On the lime-deficient soil the calcium-starved plants developed few nodules. In marked contrast to this, there were almost five times as many nodules within this soil in consequence of allowing the seedlings to grow in calcium-bearing sand and to transplant their needed calcium to the lime-deficient soil. On the neutral soil no differences in nodulation occurred as a result of either treatment to the seedlings.

In order to gain some additional information, the following chemical determinations were made: (1) the calcium content of the bean seeds, of the 10-day old seedlings grown on the calcium-deficient sand, and of those grown on calcium-bearing sand, according to a modification of McCrudden's (13) method; (2) the total dialysable base of both soils, by Bradfield's (2) method; and (3) the total dialysable calcium in these soils by the same method. The summary of these analytical data is given also in table IV, with the omission of total dialysable base.

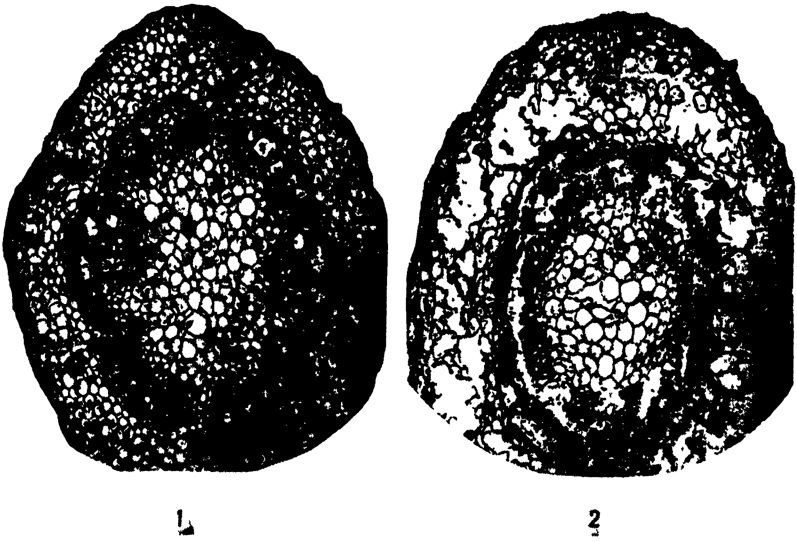
From the complete data in table IV it will be seen that the soil on which good nodulation occurred, regardless of seedling treatment, had a pH of 7.8 and gave 24.07 mg. electro-dialysable calcium per 10 gm. of soil; while the soil which gave good inoculation only on calcium-treated seedlings had a pH of 5.5 and 11.78 mg. of electro-dialysable calcium per 10 gm. of soil.

The increased nodulation of the higher calcium-containing seedlings on the calcium-deficient soil demonstrates that the presence of calcium in the plant increases nodulation of soy beans on such a soil. On the other hand, the lack of difference in nodulation of the seedlings on the more calcium-sufficient soil indicates that either the presence of calcium in the plant does not affect nodulation on such a soil, or that if it does, the calcium-starved plants take calcium from the soil soon enough to offset the measurable effect in difference in nodulation.

The increase in calcium content of the seedlings grown on the acid-extracted sand over the calcium content of the bean seeds was due to the calcium that was carried back into the acid-extracted sand by the tap water with which the sand was washed. Although the calcium content of the seedlings grown on this extracted sand was more than expected from the analysis of the seeds, the actual amount contained was but little more than half that of the seedlings grown in the calcium-bearing sand. Improved methods on this point will probably intensify the results obtained.

PART V

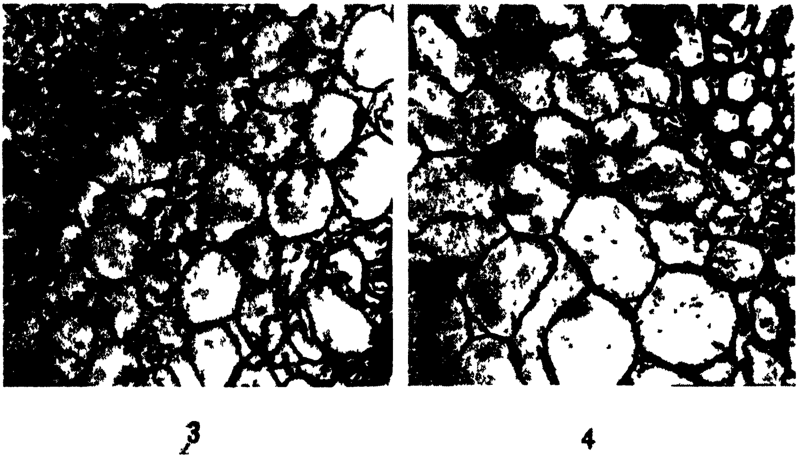
After finding that the presence of calcium within the plant increased the nodulation of soy beans on an acid lime-deficient soil, a microchemical study of the seedlings was made to locate, if possible, any histological differences in the calcium-starved and calcium-fed seedlings. Parts from both stems and roots of each of these were collected at the age of 10 days, and prepared by the usual method for sectioning in paraffin. Micro-photographs of cross-sections of the stem taken near the plant crown are reproduced in figs. 1–4. These photographs are representative sections from more than forty slides. The differences shown in the figures were the same throughout the slides. The cell walls of the calcium-starved seedlings failed to retain their shape, and apparently gave way before the microtome blade, while the cells of the calcium-fed plants stood up under the treatment and gave distinctly better sections.



FIGS. 1, 2.—Cross-section of stems of calcium-starved and calcium-bearing soy bean seedlings (10 days old): fig. 1, calcium-bearing, fig. 2, calcium-starved; ×170.

These differences were innate to the material, since the comparative sections of calcium-starved and calcium-fed seedlings were cut at the same time, and on the same microtome. The materials were fixed and processed as separate samples, but in duplicate of the same lot, so that variation in laboratory technique was reduced to a minimum. The usual macroscopic, or external differences in stems and roots in consequence of liberal and deficient calcium supply were noted before those of microscopic nature were studied. Miss DAY (6) emphasized the former for *Pisum sativum* in recent work, but she reported no significant differences in anatomical structure in these plant parts.

Both microchemical and staining methods for demonstrating calcium and pectate in micro-sections were employed in an attempt to discover any differences in the cell walls Material from 10-day old seedlings is so minute in structure, however, that as yet it has been impossible to record micro-photographically any differences noted Further work on this phase is being done, and it is expected that this observed histological difference between calcium-starved and calcium-fed soy bean seedlings can be intensified and substantiated by using seedlings more nearly deficient in calcium through growth on sand leached free of calcium with acid and distilled water, and by using seedlings of greater age to intensify their differences.



FIGS 3, 4—Cross section of stems of calcium starved and calcium-bearing soy bean seedlings (10 days old) fig 3, calcium bearing, fig 4, calcium-starved, ×750

Summary

1. An experimental study was made of the effect of calcium on nodulation of soy beans on certain acid soils, with the hope of contributing to the knowledge of the rôle calcium plays in inoculation

2. The divided root system of soy beans, grown in acid soil on one side and calcium-treated soil on the other, gave differences in nodule production to as great an extent as those produced when plants were grown wholly within these same soils. This indicates (1) that calcium plays some physiological rôle in favoring nodulation; and (2) that its effects are local or restricted in increasing the number of root infections, at least within the periods of time used in this experiment.

3. The addition of lime carbonate to an acid soil of pH 5.4, and already infected with legume organisms, gave a very marked increase in nodule production. It suggests that the effect of liming is not necessarily one of keeping alive the bacteria applied as inoculation, since in this case liming increased the number of nodules by organisms originally present in the soil. Evidently the lime carbonate exerted a physiological effect on the plants, and possibly on the organisms, to bring on the greater nodule production.

4. The addition of small amounts of calcium chloride to an acid soil (pH 5.5) increased the viability of the legume organism, *B. radicicola*, of soy beans, applied to the soil by pure cultures, and stimulated nodulation of the host plant.

5. Calcium taken up by the plant in its early growth influenced nodulation, since there was a difference in the nodulation of 10-day old calcium-starved and calcium-bearing seedlings when replanted to an already well inoculated lime-deficient soil of pH 5.4.

6. This functioning of calcium within or through the plant to produce increased nodulation may have a fundamental histological or physiological basis in the plant, since running parallel with the effect on nodulation by calcium given the seedlings, there is a distinct difference in the plant cell wall structure suggested by differences in ease of obtaining micro-sections of the 10-day old calcium-starved and calcium-bearing soy bean seedlings.

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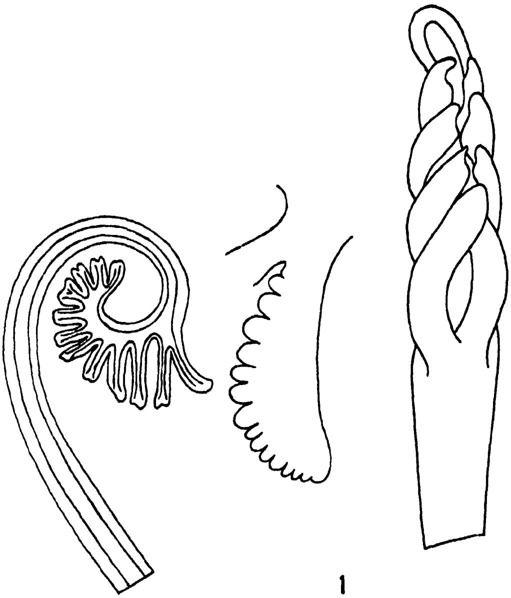
DEVELOPMENT OF SPORANGIUM IN SCHIZAEA RUPESTRIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 398

DORR RAYMOND BARTOO

(WITH TWENTY FIGURES)

The large solitary sporangia of *Schizaea rupestris* are borne in two parallel rows upon the abaxial side of the fertile pinnae. They arise in acropetal succession from marginal cells near the apex, and have already begun their development before the fertile leaves begin to unfold (fig. 1). During their early stages they become partially inclosed in two ridgelike flaps, the indusia, which are superficial outgrowths from the sporangiophore (fig. 3). Approximately twenty sporangia occur on each pinna, while the number of pinnae per leaf is from ten to twenty (fig. 2).



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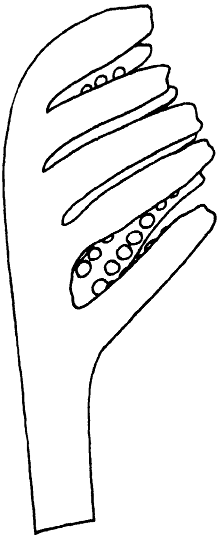

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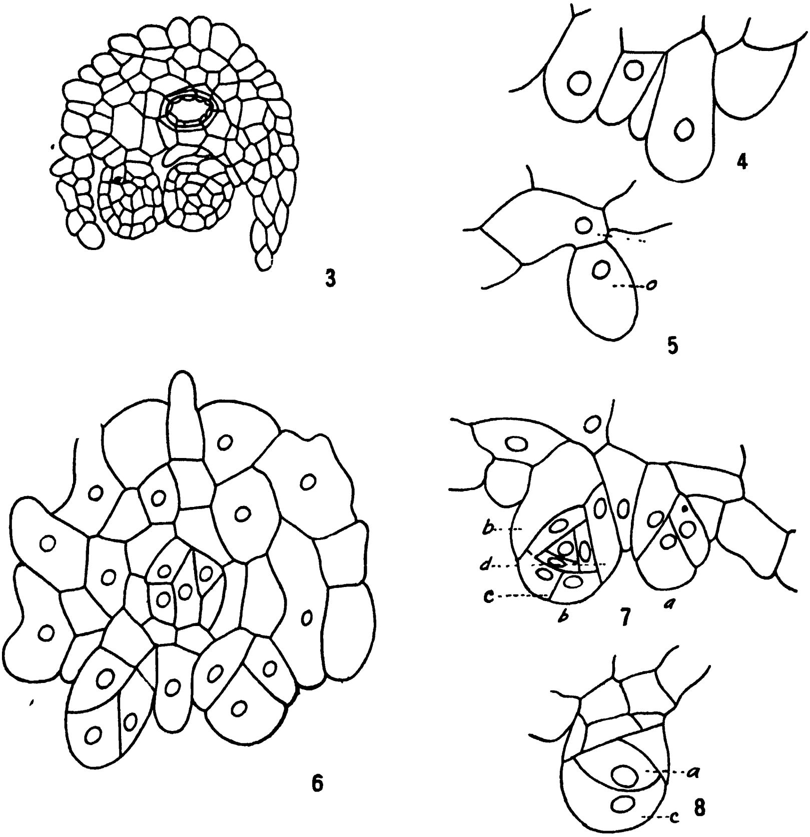
FIGS. 1, 2.—Fig. 1, early development of sporangiophore; fig. 2, later development of sporangiophore showing position of sporangia.

INITIAL AND INDUSIUM

The initial cell of the sporangium appears as a marginal cell near the apex of the pinna, and protrudes until it is hemispherical (fig. 4). Usually a second initial may be recognized in the same transverse section, thus giving two initial cells of sporangia, each of which represents the latest addition to a sporangial row (fig. 6). When first recognizable these initial cells are very close together, separated only by a single cell, as seen in either transverse or longitudinal sections.



FIGS. 3–8.—Fig. 3, transverse section of sporophyll showing sporangia and indusial flaps; fig. 4, sporangium initial; fig. 5, outer (*o*) and inner cell (*i*), developed from initial cell in fig. 4; fig. 6, transverse section of sporophyll showing early development of sporangium and indusial flaps; fig. 7, later development of sporangium; fig. 8, oblique section through young sporangium.

PRANTL[1] (6) shows that the origin of the sporangia is marginal, and that they very soon turn inward toward the lower surface. The present findings agree very closely with BOWER'S (2) illustrations and description of the origin of the sporangium initial and the subsequent development of the indusial flaps. He states:

> If sections of the pinnae be cut when very young the characteristic segmentation of the sporangia is seen to be from marginal cells, and very soon strong growth and division spring up in the adjoining cells, forming a false margin of indusium by which the sporangia are forced toward the midrib and assume a falsely superficial position. One row of them appears on each side of the midrib.

RISE OF ARCHESPORIUM

The first wall laid down in the protruding sporangial initial is level with the epidermal surface, and forms two equal cells, an outer and an inner (fig. 5 *o*, *i*). Just as in the case of other leptosporangiate ferns, the subsequent derivatives of the outer cell become the sporangium, while the inner cell takes no further part in sporangium development.

The first wall to appear in the outer cell is oblique, and meets the original transverse wall of the sporangium initial (fig. 7 *a*). A second oblique wall comes in on the opposite side and meets the first, forming a three-sided apical cell (fig. 7 *a*). A third wall then appears parallel to the first. This is followed by a periclinal wall, which gives rise to the cap cell and the archesporium (fig. 8 *c*, *a*). The cap cell very soon becomes arched over the archesporium, and by subsequent divisions gives rise to the greater part of the sporangium wall (fig. 9). The archesporium now functions as an apical cell of the dolabrate type, and from it the tapetal and sporógenous tissues arise.

APICAL CELL

The sporangium arises from a dolabrate apical cell with three cutting faces, rather than from the pyramidal apical cell of the usual fern type. Its relative dimensions and orientation are best shown in figs. 10 and 11, representing longitudinal sections cut respectively parallel and transversely to the axis of the pinna. A transverse section of the young sporangium itself gives the lens-shaped view (fig. 12). Binford (1) finds the same type of apical cell in *Lygodium*, in which the development of the sporangium is similar to that observed in the present species of *Schizaea*. On the other hand, Bower (2) states that the development of the sporangium in the Schizaeaceae follows the usual fern type in its main features, but he describes the sequence of wall formation only to the archesporium. Whether the pyramidal or the dolabrate type of apical cell prevails in the Schizaeaceae can only be determined by examination of a great number of species.

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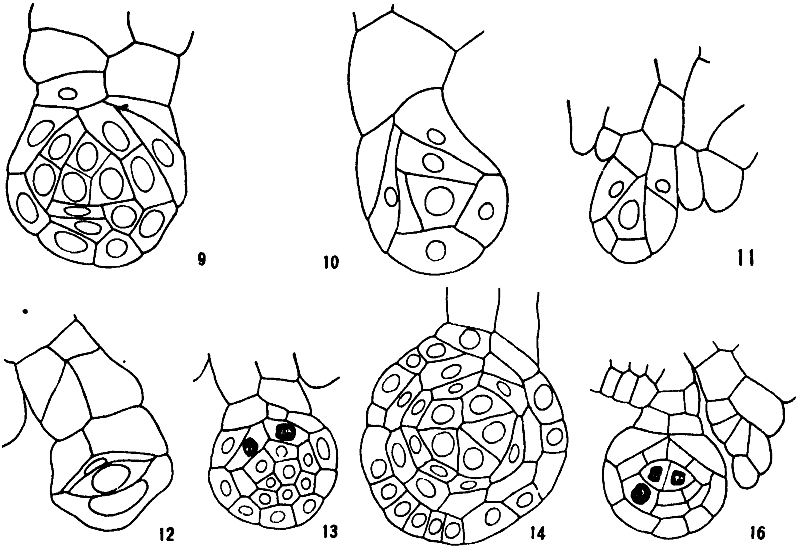
[1] By means of hand preparations, PRANTL showed that the origin of the sporangium is from the marginal cells of the pinnae of all four genera of the Schizaeaceae. His conclusion has been confirmed by BINFORD (1) in *Lygodium*, STEVENS (8) in *Aneimia*, and BOWER (3) in *Schizaea* and *Mohria*.

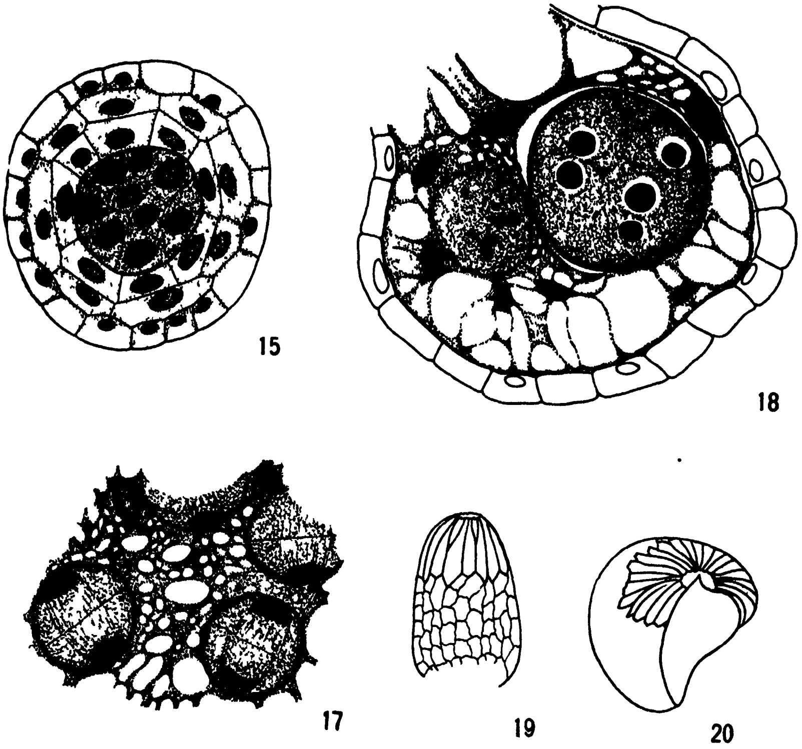


Figs. 9–14, 16.—Fig. 9, sporangium showing tapetal layer and sporogenous mass of three cells; fig. 10, longitudinal section of sporangium cut parallel to axis of pinna; fig. 11, same as fig. 10, cut transversely to axis of pinna; fig. 12, oblique section of sporangium giving two-sided view of dolabrate apical cell; fig. 13, tapetal cells of sporangium dividing to give two-layered many-celled tapetum; fig. 14, later stage of sporangium as shown in fig. 13; fig. 16, first division of primary sporogenous cell and first division in tapetal layer.

Sporangium wall

The last three cells formed from the outer cell (fig. 7 *b*, *c*, *d*) do not divide periclinally, but by subsequent anticlinal divisions give rise to a many-celled single-layered sporangium wall. As shown by fig. 13, the majority of the cells are derived from the cap cell (fig. 7 *c*).

The contents of the sporangium wall cells are only slightly granular, becoming less so as the sporangium develops, while the cells derived from the archesporium remain highly granular (fig. 14).



FIGS. 15, 17–20.—Fig. 15, still later stage showing more dense granular contents of sporogenous mass; fig. 17, spore mother cells forming tetrads; fig. 18, multinucleate condition of some of spore mother cells; fig. 19, sporangium showing terminal annulus; fig. 20, same as shown in fig. 19 after dehiscence.

TAPETUM

Just as the last three cells cut off initiate the sporangium wall, so do the next three cells cut off from the apical cell, in the same serial order, constitute the initial cells of the tapetum (fig. 7). Each divides anticlinally to form a sextet of tapetal cells, and then by periclinal divisions these six cells give rise to two layers of six cells each (figs. 13, 14). Subsequent anticlinal divisions in two planes result in a many-celled tapetum of two layers (fig. 15).

SPOROGENOUS TISSUE

Upon formation of the initial cells of the tapetum, the apical cell ceases to cut off segments and becomes the primary sporogenous cell. The first division is parallel to the long axis of the sporangium, resulting in two equal cells (fig. 16). Three successive divisions follow, giving rise to sixteen spore mother cells. Occasionally eighteen to twenty spore mother cells were observed. It was noted that the first divisions of the sporogenous tissue were simultaneous with the first divisions of the tapetal initials (fig. 16).

Although at the time of spore mother cell formation all the cells within the sporangium wall have dense granular contents and large nuclei, the cells of the sporogenous tissue are differentiated from the surrounding tapetal cells by their deeper stain (fig. 15); in fact, at no time is it difficult to distinguish the denser sporogenous mass.

As was observed by BINFORD, subsequent to the formation of spore mother cells there is an enormous increase in the sporogenous mass. The dissolution of the middle lamellae and the consequent rounding off of the spore mother cells go on simultaneously with the breaking down of the massive tapetum. The formation of tetrads and spores quickly follows (fig. 17). The nuclei and cytoplasm of the tapetum flow into the sporogenous mass so that the young spores are surrounded by an abundance of food. STEVENS (8) has shown that the movement of cytoplasm actually takes place, the tapetal plasmodium creeping in between the spores and holding each one separately imbedded. According to BINFORD there is a diversion of food material from tapetal to spore formation. First there is a comparatively small amount of growth in the sporogenous mass, with a large growth of the sporangium wall and tapetum (fig. 15), but after the spore mother cells have rounded off and become imbedded in the tapetal plasmodium, growth is rapid and the spore mass becomes comparatively large. Thus the nutrition which is first stored in the rapidly growing tapetal layers is later transferred to the rapidly growing sporogenous mass.

MULTINUCLEATE SPOROGENOUS CELLS

It has already been stated that sixteen is the usual number of spore mother cells. Not infrequently, however, wall formation in the sporogenous mass fails to follow nuclear division. Thus sporangia were occasionally found which contained from two to fourteen spore mother cells, some or all of which were multinucleate. No cases were observed where there was a failure of wall formation immediately following nuclear division of the archesporium. After the second nuclear division one of the walls sometimes fails, however, resulting in a sporogenous mass of three cells, one of which is binucleate. The ultimate result of succeeding nuclear divisions is a sporogenous mass of nine mother cells, one of which contains eight nuclei and is equal in mass to the sum of the other eight uninucleate spore mother cells. It would seem that successive nuclear divisions take place too rapidly for walls to form. Or after the failure of wall formation has once occurred it is probable that the protoplasmic mass is too great for any subsequent nuclear division to form a wall. However, four successive nuclear divisions occur in the sporogenous mass just as in the usual condition of wall formation. Other sporangia were observed in which wall formation had failed to occur after the second successive nuclear division, resulting in one or more four-nucleate spore mother cells. Similarly there were observed cases where wall formation had failed in the third successive nuclear division, giving one or more binucleate spore mother cells. No cases were observed in which the sporangia did not contain some uninucleate spore mother cells; thus wall formation had taken place in at least part of the sporogenous mass (fig. 18).

STALK

The short stalk, consisting of two or three cells, is derived from the first cells cut off from the sporangium initial (fig. 11). It often remains a single cell in length but sometimes one or more of the stalk cells undergo cell division (fig. 13).

ANNULUS

The development of the annulus which arises from the cap cell (fig. 8 *c*) has been completely worked out by PRANTL (6). By divisions of the cap cell fourteen or more elongated ring cells develop (figs. 19, 20). A single three- or five-angled "plate" cell, having one of its angles directed toward the stomium, is formed at the apex of the sporangium (fig. 20).

RELATIONSHIPS

While the nature of the present investigation does not warrant a discussion of the primitive and advanced characters of the Schizaeaceae as a whole, it may be worth while to enumerate some of the outstanding characters of *Schizaea* itself. It is primitive in its simple leaves, its apparent dichotomy, and its open venation. On the other hand, its definite annulus and reduced output of spores (prevailingly 128 per sporangium)[2] relate it to the more advanced leptosporangiate ferns. The greatest reduction of spore output apparently takes place in the presently investigated species (64 per sporangium).

The relationship of the Schizaeaceae to the Marsileaceae, as supported by CAMPBELL (5), is an interesting one. He observed the remarkable similarity between the structure of the very young sporocarp of *Marsilea* and the fertile leaf segment of *Schizaea*, and also noted that the "filiform leaf of *Pilularia* finds its exact counterpart in the sterile leaves of *S. pusilla*."

BOWER (2) has made a systematic comparison of the Marsileaceae and the Schizaeaceae, and by way of summary states:

> The result of this comparative analysis is to render support to CAMPBELL'S recognition of a real relationship of Marsileaceae to Schizaeaceae, its similarity extending along the whole line of comparison. . . . . In any case the relationship to the Schizaeaceae seems beyond doubt. The Marsileaceae may be held to be a family of the same fundamental type, though specialized for aquatic life, and heterosporous. But it does not seem possible to link them definitely with any single genus of the Schizaeaceae; the similarities are, however, closest to *Schizaea* and *Anemiorrhiza.* In support of the comparison it may be noted that *S. rupestris* is to be found growing in a very moist condition, under dripping rocks, in the Blue Mountains, New South Wales, as a veritable hydropterid in habit.

The present investigation renders even greater support to the proposed relationship between the two families, and, as already suggested by BOWER (2), the similarities of Marsileaceae to the genus *Schizaea* are greatest. The species of *Schizaea* which lies nearest to *Pilularia* is probably *S. rupestris*. In the first place, *S. rupestris* has made the greatest advance of all observed species in the reduction of the number of spores per sporangium; 64 as compared with the prevailing number of 128. This reduced number of spores has reached the upper limit of *Pilularia* (64–32 per microsporangium). Secondly, the multinucleate condition of the spore mother cells of *S. rupestris* may be interpreted as a step toward heterospory (fig. 18). After nuclear division, walls have failed to form and in some cases there is evidence of the disintegration of nuclei within the multinucleate cells. Occasionally four small elongated nuclei are so placed with respect to one another that it would indicate a feeble attempt at tetrad formation (fig. 18).

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[2] BOWER (2) found 128 typical for species of *Lygodium*, *Aneimia*, and *Mohria;* BINFORD (1) found 242–256 by actual count in *L. circinatum;* TANSLEY (9) counted 90–115 spores per sporangium in *S. malaccana.*

WILLIAMSON and SCOTT (10) conclude from their investigation of *Calamostachy casheana* that heterospory was reached by the abortion of spores in certain sporangia, the remaining, because of excessive nutrition, becoming "specialized spores." SHATTUCK (7), by controlling cultures of *Marsilia*, obtained all degrees of abortion in the microsporangium. When all the microspores except one aborted it was "about sixteen times as large as a normal microspore," and had acquired other "certain megaspore characters."

The spore output per sporangium in *S. rupestris* has reached the upper limit of that of the microsporangium of *Pilularia*. In the megasporangium of *Pilularia* only one of the spore mother cells develops to the tetrad stage, and only one of the four daughter cells becomes a mature megaspore. It seems probable that the failure of wall formation followed by the abortion of nuclei within the sporangium of *S. rupestris* has become a constant feature in the megasporangium of *Pilularia*. There is little doubt that future investigations upon the origins and development of the tissues of the plant bodies will establish a real relationship between the homosporous Schizaeaceae and the heterosporous Marsileaceae.

Summary

1. The origin of the large solitary sporangium is marginal.

2. The sporangium wall and tapetum arise from segments cut from a dolabrate apical cell.

3. The primary sporogenous cell by three successive divisions gives rise to sixteen spore mother cells.

4. Subsequent to the formation of the spore mother cells and the tapetal plasmodium there is rapid increase in the sporogenous mass.

5. A striking feature is the formation of multinucleate spore mother cells, which is not uncommon.

6. Two features render additional support to the relationship of Schizaeaceae to Marsileaceae proposed by CAMPBELL and supported by BOWER: (*a*) The spore output of *S. rupestris* has advanced to 64 per sporangium as compared with the prevailing number of 128 in most species. This reduction in spore output has reached the upper limit of *Pilularia* (64–32). (*b*) Multinucleate spore mother cells resulting from a failure of wall formation following nuclear division is a step toward the heterosporous condition of the Marsileaceae.

Acknowledgments are due Professor W. J. G. LAND, under whose direction this investigation has been conducted. Helpful suggestions have also been given by Professor C. J. CHAMBERLAIN. Thanks are due to Mr. PATRICK BROUGH of the University of Sydney, who collected and fixed the material used.

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FIELD OBSERVATIONS ON PERUVIAN HEPATICAE

GEO. S. BRYAN

(WITH SIX FIGURES)

In 1923 I went as a member of the Botanical Expedition of the Field Museum of Natural History, Chicago, to the Peruvian Andes, to study and collect the Hepaticae of that region. My whole attention was to be given to the cryptogams, while Dr. J. FRANCIS MACBRIDE was to devote himself to the phanerogams. This paper is intended as a preliminary report on the Hepaticae of the regions visited.

Field methods

In order that the specimens of the Hepaticae might be used for morphological study, it was decided to employ the standard combination of chromic and acetic acids (in the proportions 1 gm. chromic acid, 1 cc. glacial acetic acid, 400 cc. water) as a killing and preserving agent. The following equipment, therefore, was carried on the expedition: small phials each containing 10 gm. of chromic acid crystals, two 100 cc. bottles of glacial acetic acid, a 100 cc. glass graduate, and a 1000 cc. bottle. All were carefully packed in special containers. The killing fluid was made up in camp as there was need for it, rain water being collected and employed wherever possible. Several hundred 125 cc. wide-mouthed bottles were used as containers for the specimens. These bottles were packed in light but strong boxes, about eighty bottles in a box, each bottle being carefully padded. The corks were waxed and tied securely down by twine.

Our experience would seem to indicate that glass bottles can be used on expeditions as containers for specimens provided proper care is taken in packing them. The collected specimens remained in the killing fluid for nearly a year without any apparent damage or deterioration. Samples of most of the Hepaticae were sent to Professor ALEXANDER W. EVANS, Yale University, who has kindly provided a preliminary check list of the genera, and in some cases of the species. I take this opportunity of acknowledging my indebtedness to Professor EVANS for this courtesy.

Field stations

CHOSICA.—The expedition landed at Callao on March 6, and soon began work in the vicinity of Chosica, which lies in the foothills of the Andes about 25 miles from Lima. The elevation of Chosica is 2800 feet. The rainfall in this region is generally scanty at all times of the year, consequently the high hills are barren except for cacti, and on some slopes bromeliads. In sheltered pockets and ravines a few shrubs, grasses, and other flowering plants eke out a precarious existence. It was not surprising that field studies failed to locate any liverworts in this region.

MATUCANA.—From Chosica our base of operations was moved into the mountains, to the village of Matucana, with elevation of about 8000 feet. This is an area of almost daily rainfall at this time of the year (March). The contrast with the barren hills about Chosica was marked. Here the steep rugged slopes of the mountains were green with grasses, shrubs, and small flowering plants. Heliotrope was abundant and in full bloom. On exposed and rocky areas cacti and bromeliads were conspicuous. Lichens were everywhere abundant.

Careful search revealed only two liverworts in this region. In various localities, but chiefly in those shaded by rocks or shrubs, grew small mats of *Plagiochasma rupestre* (Forst.) Steph. The production of sex organs had practically ceased, and large numbers of sporophytes, many of them mature, were in evidence. In similar habitats and sometimes associated with *Plagiochasma* was found a species of *Targionia*. It had also passed the stage of sex organ production, and maturing sporophytes were numerous.

RIO BLANCO.—Rio Blanco is 20 miles farther into the mountains, and has an elevation of 11,300 feet (fig. 1). Among the conspicuous flowering plants of this region were long grasses, nasturtiums that clambered over rocks and shrubs, an oxalis with a thick woody stem, a large purple geranium, and, on low areas near the brooks, yellow masses of *Senecio*. The liverworts were also more plentiful here. In damp shaded places grew fine mats of *Plagiochasma rupestre*, and *Targionia* was almost as abundant. At this higher altitude many of the plants were still producing sex organs, and only a few mature sporophytes were visible.

On a sheltered dripping bank, at an altitude of about 12,000 feet, a *Marchantia* was found which has been identified by Professor EVANS as *M. plicata* Nees & Mont. (fig. 2). The plants were smooth in appearance, olive green in color, and were large and vigorous, some of the branches measuring nearly 2 inches across. Many of the plants bore young female receptacles which, like the thalli, were also large. No male receptacles could be located, but that male plants were undoubtedly present was indicated by three greatly enlarged female receptacles crowded with almost mature sporophytes.

FIG 1—Difficult terrain at Rio Blanco

In the shade of shrubs and bushes, and on bare spots among tall grasses, at an elevation of more than 12,000 feet, were discovered scattered plants of a very delicate *Fossombronia*, *F. crassifolia* Spruce. Many of the plants bore mature sporophytes, the round black capsules on relatively long slender stalks being quite conspicuous.

While studying the plants of *Fossombronia* under a large reading glass, I came by chance on a very small thalloid liverwort bearing several sporophytes on a short-stalked receptacle. A considerable area in the vicinity of the discovery and other likely habitats were carefully searched with the reading glass, but only about half a dozen specimens were secured. This small liverwort has proved to be *Sauteria berteroana* Mont., which hitherto has been known only from

Chili. It is possible that this plant has a somewhat wide distribution along the high Andes, since its small size might readily cause it to be overlooked by collectors.

CERRO DE PASCO.—This location was reached by crossing the backbone of the great western range of mountains. The rolling hills about Cerro, which have an elevation of about 15,000 feet, are for the most part clothed with short grasses. Low patches of a woolly cactus form "mountain sheep," and give a curious appearance to some of the slopes (fig. 3). In a cuplike depression among the hills more plants of *Fossombronia crassifolia* were located, but the most interesting event was the discovery of small patches of a *Riccia* on the wet soil in the bottom of the depression. A field examination with a traveling microscope showed that the plants were clearly dioecious. Professor EVANS is of the opinion that this *Riccia* is probably an undescribed species close to the North American *R. austini*. It requires further study.

FIG. 2.—*Marchantia plicata* Nees & Mont. at Rio Blanco

HUANUCO.—This town, 65 miles northeast of the previous location, lies in a valley at the head of the Huallaga River, one of the tributaries of the Amazon. The elevation is about 7000 feet. The rainfall in this particular region is relatively light, and no liverworts were found except some patches of sterile *Lunularia* on a sheltered moist bank.

MITO.—This village lies 18 miles northwest of Huanuco, at an elevation of about 8500 feet. To the north east the mountain range makes a great horseshoe bend which is furrowed by three canyons. The rainfall is much greater in this bend than in any of the adjacent regions, and the low montaña[1] which clothes the three canyons proved to be an excellent region for collecting. This area yielded more than forty species of ferns, many lichens and mosses, and a variety of liverworts.

On damp shaded soil in the bottom of the most westerly canyon were small patches of *Asterella macropoda* (Spruce)Evans. Many of these plants bore mature sporophytes, the maximum number on a receptacle being four. Later in other localities excellent specimens were found which showed that the species is monoecious, and that antheridia are borne on slender lateral branches which extend on the under side of the thallus from the midrib to the margin.

FIG. 3.—Woolly cactus at Cerro de Pasco

Farther up this canyon, on a wet shaded bank of the stream was a mat of *Noteroclada confluens* Tayl., a few of the plants bearing mature sporophytes; and in the immediate vicinity of *Noteroclada* were plants of *Symphyogyna braziliensis* Nees, some of which bore sex organs and others young sporophytes. Another *Symphyogyna, S. sinuata* (Sw.) Nees & Mont., was found growing nearby in considerable quantity in short grass on a wet slope of the canyon. This variable species was here characterized by a stout midrib and delicate but deeply cut lobes which, in some of the specimens, were so pronounced that they might equally well be called leaves.

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[1] Mountain forest of the Andes.

On many of the shaded rocks in the upper reaches of the canyon, in the precipitous stream bed, and on dripping faces of the canyon wall, were found great masses of *Dumortiera hirsuta* (Sw.) Nees. The plants showed all stages of development of antheridial and archegonial receptacles, and even a few mature sporophytes. *Dumortiera* in some localities is practically an aquatic liverwort; and in another locality it was found thriving on the face of a low cliff, although submerged by a sheet of water that seeped over it. It was also found near a densely shaded waterfall, where it grew on the rocks beside the fall, kept constantly wet by the spray.

In a wet but rather open area beside the stream bed was found a straggling mat of *Megaceros*, and nearby on a deeply shaded clay bank at the foot of a great dripping rock were small patches of *Clevea andina* Spruce. The thallus of *Clevea* here collected is thin, delicate, and pale green in color. A great many of the plants bore mature sporophytes.

Near the more open, basal end of the canyon a small marshy area was practically covered by *Marchantia plicata*. The plants were not so large as those at Rio Blanco, but bore receptacles in all stages of development. On moist but rather open ground was found another species of *Marchantia* which I shall refer to as no. 338, its collection number. Professor EVANS has examined the plant and thinks that it is probably a new species resembling somewhat the West Indian *M. breviloba*. Abundant material was collected in various other localities to be described later, and the morphology of the plant is now being studied. Two other liverworts, an *Anthoceros* and a *Lunularia*, only male plants of the latter being found, complete the list of Hepaticae collected from the soil and rocks about Mito.

The low montaña of the canyons yielded several epiphytic liverworts, the most abundant being species (at least three) of *Metzgeria* which festooned the branches of the trees and bushes. These species of *Metzgeria* were in ideal condition for collection, since they bore sex organs as well as practically mature sporophytes. *Brachiole-*

jeunia laxifolia (Tayl.) Schiffn., a *Plagiochila*, and male plants of a large *Porella* were also somewhat common.

TRAIL TO POZUZO.—Early in May we began the trail to Pozuzo, a small village about 100 miles away across the easternmost range of the mountains and near the navigable waters of the Rio Pachitea. We planned to camp along the trail, stopping and collecting wherever it seemed worth while.

The first few days of travel had little of interest in the way of liverworts, as the mountain slopes were almost as dry as those about Huanuco. In the vicinity of the village of Panao, however, on the side walls of a deep and narrow gully, were found large sheets of *Asterella venosa* (Lehm & Lindenb.) Evans, with receptacles in various stages of development; and in a canyon in the same locality an abundance of *Symphyogyna braziliensis*. *Marchantia* no. 338, bearing mature sporophytes, was collected in considerable quantities beside the trail near Panao, and again below the pueblo of Chaglla which lies about 9 miles beyond Panao.

About Piedre la Grande was found a mixture of grassland and low brush in which a species of *Botrichium* grew in great abundance. In this region only a few liverworts could be found. From a small area of montaña in a ravine came two species of an epiphytic *Plagiochila*, and a *Porella*, all of which bore sporophytes. On the clay banks of a little brook above the trail were scattered plants of *Symphyogyna braziliensis*, which had also been collected at Mito and Panao; and on the steep and deeply shaded muddy banks of the same brook close to the trail were found scattered patches of *Plagiochasma intermedium* Lindenb. & Gottsche. Professor EVANS states that this is the first time the species has been reported from South America. Some of the specimen collected bore sex organs and others mature sporophytes. Although a careful search was made in a number of likely habitats in the vicinity no other plants could be found.

MUÑA.—In the dry scrub leading up to the montaña a great wealth of lichens and probably two species of *Porella* festooned the bushes and low spreading trees. In the cool moist montaña of the canyons were considerable quantities of *Frullania campanensis* Spruce, but *Frullania hians* (Lehm & Lindenb.) Nees & Mont. was found but rarely and only in small mats. Both species bore practically mature sporophytes. A species of *Metzgeria* as yet undetermined, and *Dicranolejeunea axillaris* (Nees & Mont.) Schiffn. were abundant on the trees in one of the canyons. Both plants had numerous mature sporophytes.

In a canyon about a mile south of Muña *Dumortiera hirsuta* was again found in its usual moist habitat. Associated with *Dumortiera* or in similar habitats were fine mats of *Monoclea gottschei* Lindb. The plants bore mature sex organs and sporophytes in various stages of development. To the east and at an elevation of about 1000 feet above Muña the scrub passes into a magnificent tropical forest with a fine display of tree ferns and a wealth of epiphytes. In this montaña were found, on decaying sticks and leaves, patches of *Megaceros*, and in some rather open places areas of *Anthoceros*. On decaying logs and on mats of dead leaves *Riccardia pinguis* (L.) S.F. Gray was common. Sex organs and sporophytes were abundant on these plants. The list of Hepaticae from Muña is concluded with two species of *Symphyogyna*. A small patch of *S. leptothelia* Tayl. was located on a shaded clay bank in the montaña; and not far away on a wet, grassy clay bank were abundant plants of *S. sinuata*. Both species bore mature sporophytes. The plants of *S. sinuata* were characterized by wavy margins, but no deeply lobed specimens could be found.

TAMBO DE VACA.—On June 7 camp was moved 9 miles farther to the east and close to the summit of the great eastern range of the Andes. This tropical forest, beginning at an elevation of about 1000 feet above Muña, extends far on up the mountain, gradually passing near the top into a montaña of low, widely branching trees (fig. 4). Near the crest the montaña is interrupted here and there by small areas of grassland and meadow, and is known by the appropriate name of Tambo de Vaca. The elevation is nearly 13,000 feet. The region about Tambo de Vaca contained a great wealth of epiphytes. The low spreading trees were literally clothed with a variety of ferns, mosses, lichens, and leafy liverworts (fig. 5). The reason for this wonderful display was evident. For much of the year the warm moisture-laden winds from the east drench the top of this eastern range with dense mists or heavy rains, and even in the dry season the crest is enveloped in swirling clouds and light rains are not infrequent.

FIG. 4.—Low montaña at Tambo de Vaca

FIG. 5.—At edge of meadow in low montaña; trees covered with ferns, lichens, liverworts, etc.

Unfortunately a box containing, among other things, dried specimens of leafy liverworts from this region was lost in transit to New York, consequently I am able to give but an incomplete list of the Hepaticae from Tambo. One of the most abundant epiphytic liverworts was *Frullania brasiliensis* Raddi (fig. 6). In some localities it dominated the epiphytes and in certain localities trees were found entirely covered by it. *Radula ramulina* Tayl. was also quite abundant, but seemed to prefer shaded and less exposed habitats. Among the less common forms were a *Plagiochila*, apparently quite different from the two species found at Piedre la Grande, and a *Trichocolea*. The latter bore mature sporophytes only. *Metzgeria filicina* Tayl. occurred in small amounts in well shaded habitats. Near the trail, at an elevation of about 12,000 feet, on a shaded, wet clay bank and partially hidden in a scanty growth of grass, were some fine specimens of *Symphyogyna brongniartii* Mont. All the plants examined were deeply lobed, and in nearly all cases the lobing extended to the thick stemlike midrib. Superficially these lobes are indistinguishable from leaves, and are exceedingly brittle. The plants bore sporophytes in varying stages of maturity, and it was interesting to note that the sporophytes were in most cases about twice the size of those of *S. sinuata* which was collected in the same vicinity but at a lower altitude. *S. sinuata* and *S. brongniartii* may not be specifically distinct, but certainly the specimens found in this region have very different characters.

FIG. 6.—Epiphytic vegetation within low montaña

Riccardia pinguis, which was quite abundant in the montaña above Muña, was found to extend its range up the mountain to an elevation of about 12,000 feet. *Asterella macropoda* and a *Marchantia*, probably *M. chenopoda* L., were found near the crest of the mountain at an elevation of nearly 13,000 feet.

The vegetation of the high eastern slopes, which were next traversed, was quite similar to that of the western. First low trees were encountered, covered with ferns, mosses, lichens, and leafy liverworts. There were also the same familiar small areas of grassland and meadow. But the most striking new feature was the luxuriant growth of bamboo, small areas of which had been observed on the high western slopes. The liverworts were disappointingly the same, only one new form being found on the first day's journey, a species of *Dendroceros* which was somewhat abundant in one particular locality near the trail, but bore neither sex organs nor sporophytes.

In the vicinity of Cushi two liverworts were quite common, one being *Asterella macropoda*, already found in various other localities, the other was *Marchantia chenopoda*. In one or two places along the trail near Cushi I observed the now familiar *Dumortiera hirsuta*, *Monoclea gottschei*, and *Riccardia pinguis*, and took a few samples of each.

Near the settlement of Pozuzo fine specimens of *Monoclea* and *Dumortiera* were found in abundance. In relatively dry but shaded habitats were considerable quantities of a very small, delicate *Marchantia* which Professor EVANS has identified as *M. chenopoda*. The plants were all uniformly small and delicate, being much less than half the size of the more characteristic form found at higher elevations. The structure and development of the sporophyte of this small form are being studied and contrasted with *M. polymorpha* in this laboratory, and interesting differences have been found.

TRAIL TO CHINCHAO.—On a densely shaded bank near Chinchao were numerous plants of *Pallavicinia erythropa* (Gottsche) Steph. At this late season of the year they bore sporophytes in various stages of development. In this vicinity was again observed the small delicate form of *Marchantia chenopoda* which had been found near Pozuzo.

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CURRENT LITERATURE

BOOK REVIEWS

Plant succession and indicators

A volume has just appeared by CLEMENTS[1] which is a combined and condensed edition of *Plant succession*, issued in 1916, and *Plant indicators*, issued in 1920. The impulse for this condensed edition comes from the fact that the supply of original editions of both works is exhausted. Essentially no new material has been added, and considerable portions of the text have been omitted, notably chapters X and XI of the first mentioned book, dealing with succession in North America and Eurasia; also chapters XII and XIV on paleoecology. Chapter IV of the second book, dealing with climax formations of western North America, is also omitted. These particular omissions are made because it is anticipated that in the near future extensive treatises will be published by the authors dealing with these subjects in enlarged style. In order to save additional space, a number of the plates and figures of the former works are omitted, also the indexes. The bibliography is very much reduced. There is, however, an ample table of contents, so that it is not difficult to find any particular subject desired.—H. C. COWLES.

Physiological basis of drought resistance

The excellent monograph of MAXIMOV[2] on the relations of the plant to water, which has just been published in English translation, deserves a wide reading. Written by an eminent Russian plant physiologist and edited by a well-known English ecologist (whose obituary notice we regret to see in the preface), the book has an authoritative point of view in the synthesized field which it covers.

The first half of the work deals with the physiology of the intake and outgo of water by the plant, and with the factors which control those processes. These chapters discuss these matters concisely and critically, and are up-to-date in their treatment. But while they are valuable because they bring all the material conveniently together, they are not so notable as the chapters of the second half of the book, wherein the nature of the drought resistance is considered. Here is

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[1] CLEMENTS, F. E., and CLEMENTS, EDITH S., Plant succession and indicators. pp. 460. *pls. 44. figs. 24.* New York: H. W. Wilson Co. 1928. $8.50.

[2] MAXIMOV, N. A., The plant in relation to water: a study of the physiological basis of drought resistance. Ed. by R. H. YAPP. 8vo. pp. 451. *figs. 46.* London: George Allen and Unwin Ltd. 1929. *21s.* Macmillan Co. 1929. $6.50.

MAXIMOV's real contribution. He offers convincing evidence that the true criterion of xerophytism is the ability of the plant to recover again after complete wilting, and not the ability to prevent that wilting by reducing the rate of transpiration. "All influences which result in greatly increased loss of water by the plant, or a restricted supply of water to the developing leaves, lead to essentially similar changes of leaf structure." These changes ("xeromorphic") tend to facilitate water supply and also to increase gas exchange, and therefore xeromorphous plants have higher, rather than lower, rates of transpiration and photosynthesis.

Xerophytes do not seem to be able to use water from drier soil than other plants. They simply wilt when they can no longer obtain enough water, and are able to recover again later. Xerophytic characters are (1) anatomically: decrease in size of all cells, thickening of cell walls, strong development of palisade mesophyll, denser network of veins, and increased number of stomata per unit area; and (2) physiologically: increase in intensity of photosynthesis and transpiration, in osmotic pressure, and in capacity to endure wilting. TUMANOV has found some evidence that plants may be "hardened" against drought in ways analogous to those of hardening against cold in use now, so that "drought during early stages may be beneficial"! Another concept of great value is that of a critical period of drought for various kinds of crops. Not the total water available, but the time in the development of the plant at which the supply is deficient, seems often to be the determining factor.

Throughout the whole book one is continually aware of the great care which the author has taken to collate all work bearing on the subject, to present data whenever it would clarify the argument to do so, and to analyze data and arguments critically. Indeed, the reader may sometimes decide that the author is not wholly justified in his conclusions, so fully does he present all the evidence. But his reasoning is clear, with rare exceptions. When he takes issue with LIVINGSTON as to the value of the decreasing water content of the leaf in curtailing transpiration, his zeal for his own explanation makes him less clear than usual as to the relative importance of the various regulatory mechanisms in different situations. Consequently we read that "LIVINGSTON's concept of 'incipient drying' is of very limited importance," whereas "a similar view was put forward by SRESNEVSKI some years previously," and "this purely physical regulation of water loss . . . . . established by SRESNEVSKI is of extreme importance," statements whose context does not alter their apparent meanings as here given. MAXIMOV thinks unconsciously only in terms of cases of dry soils, but even here the immediate cause of transpiration decrease must be a shortage of water in the leaf cells. His theory of curtailment of transpiration by shortage of water in the root cells seems to the reviewer to differ little from LIVINGSTON'S in the final analysis. But the instance cited is exceptional, and the book is also singularly free from typographical errors.

The inclusion of the great amount of Russian work in this field, normally quite unavailable to English-speaking readers, is a valuable feature of this book;

but all American, English, German, and French work up to 1925 (when the Russian edition was published) with no notable exceptions has been taken into account, and the more important work even up to 1928 has been included in most phases of the subject. The result is an up-to-date, authoritative presentation of an important phase of plant physiology and ecology, with many practical applications suggested in the field of drought resistance.

An extensive bibliography is appended and an excellent index.—H. S. WOLFE.

Phytopathology

Recent volumes by OWENS[3] and BROOKS[4] indicate that the intellectual climate of phytopathology in the United States and in England has definitely changed. Although phytopathology on the continent always considered its content to include any disease and undesirable anomaly no matter how induced, American and English usage irrationally has virtually restricted the content of phytopathology to diseases and anomalies caused by bacteria, certain myxomycetes, fungi, and a few animals such as nematodes. In other words, phytopathology was essentially applied phytobacteriology and phytomycology.

A different trend was indicated by WHETZEL's laboratory manual[5] and later by HEALD's excellent manual.[6] With the publication of the volumes by OWENS and by BROOKS, the English language has two more books in phytopathology which, while they emphasize the diseases caused by bacteria and fungi, at least indicate that non-parasitic diseases and diseases caused by algae, for example, are a part of the content of phytopathology. Neither book goes all the way to include the abnormalities caused by insects. Both books preface their special parts with a discussion of the fundamental principles that form the basis of the science. Even though these discussions are far too brief and incomplete, it is to be hoped that they augur that phytopathologists are beginning to look upon their field as more than a congeries of individual diseases.

OWENS' book, like HEALD'S, was written with the need of his classes in mind. Consequently one misses, even in the special parts, detailed discussion of diseases that occur in the south. The arrangement of the special diseases by OWENS is not as sound, considered from the point of phytopathology as a science, as that by BROOKS. There is a definite pedagogical gain in taking up first the non-parasitic diseases. A brief presentation of the structure and life histories of the classes of parasites would have enhanced the value of OWENS' volume as a textbook. However, the book fills a real need, especially for agricultural students, who after all, are not so much interested in the science per se as they are in the diagnosis and control of plant diseases.

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[3] OWENS, C. E., Principles of plant pathology. pp. xii+629. *figs. 222.* New York: John Wiley and Sons. 1928.

[4] BROOKS, F. J., Plant diseases. pp. vii+386. *figs. 62.* Oxford Press. 1928.

[5] BOT. GAZ. **81**:470. 1926.

[6] BOT. GAZ. **82**:443. 1926.

BROOKS' volume is essentially a brief discussion of the diseases which occur in crops in the British Empire. It is surprising that such a volume has not appeared before this. The discussions, in the main, are brief descriptions of the individual diseases. It is gratifying that this volume has come from one of the students of H. MARSHALL WARD.—G. K. K. LINK.

Control of pathogens

An excellent volume has been prepared by TRAPPMAN[7] on control of the pathogens (Pflanzen Schädlinge) of plants, in which he includes all living agents, parasitic and non-parasitic, which injure plants. Considerable attention is devoted to control of those factors which affect the susceptibility and resistance of plants to attack by injurious agents, and the occurrence and abundance of these. In doing this he incidentally considers control of non-parasitic diseases. The introductory chapters are devoted to (1) significance and aim of plant protection; (2) concepts of disease and injury; (3) causes of injuries (in the sense of damage); (4) parasite and host; (5) disease symptoms; (6) occurrence and distribution of pathogens. Control is treated under the main headings: cultural practices; biological methods; technical methods using physical means; technical methods using chemical means; evaluation of these methods; and organization of control.

The influence of MORSTATT's volume is very marked in the introductory chapters. Account has been taken to a surprising extent of plant disease control methods and research in the United States. The volume is a timely and useful compilation of widely scattered but highly important data for anyone concerned with the control of plant diseases. It is significant that it appeared as the 8th volume in the series *Chemie und Technik der Gegenwart*.—G. K. K. LINK.

Manual of microbiology

The volume by FRED and WAKSMAN[8] is a useful addition to the literature on the laboratory phases of microbiology. It gives adequate and clear directions for the standard laboratory manipulations, such as preparation and formulas for culture media, methods and formulas of staining, qualitative and quantitative methods of analysis, and the study of microorganisms in the soil.—G. K. K. LINK.

NOTES FOR STUDENTS

Protoplasmic ether-soluble constituents.—An interesting and valuable study of the ether-soluble constituents of cabbage leaf cytoplasm has been made by CHIBNALL and CHANNON, who began to publish on this subject[9] about two years ago. After preparation of the leaf cytoplasm from green unheaded cabbage as a solid cytoplasmic mass, it was extracted with anhydrous ether, 149 gm. of cytoplasm yielding about 17.6 gm. of ether-soluble extractives.

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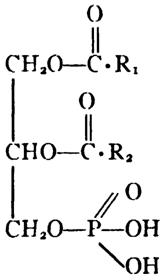
[7] TRAPPMAN, W., Schädlingsbekämpfung. 8vo. pp. viii+446. *figs. 68.* Leipzig: S. Hirzel. 1927.

[8] FRED, E. B., and WAKSMAN, S. A., Laboratory manual of general microbiology. New York: McGraw-Hill. pp. viii+145. *figs. 19.* 1929.

[9] CHIBNALL, A. C., and CHANNON, H. J., The ether-soluble substances of cabbage leaf cytoplasm. I. Preparation and general characters. Biochem. Jour. 22:225–232. 1927.

In general, the ratio of protein in the cytoplasm to the ether-soluble extract was about 3:1. About 42 per cent of the fatty material contains phosphorus, and 58 per cent is phosphorus-free. Approximately 17 per cent of the extract is unsaponifiable, and fatty acids make up about 23 per cent of the total. The phosphorus-containing fraction[10] was precipitated with acetone. Examination of this acetone-precipitable fraction showed that there were no phospholipins present, but that all the phosphorus was tied up with calcium, glycerol, and fatty acids. The main constituent was found to be the Ca salt of diglyceridephosphoric acid, with some Ca monoglyceride phosphate.

A more detailed examination[11] of the Ca salts of diglyceridephosphoric acid led to the conclusion that the free acids and its salts should be classed, according to Thudichum's early classification, as phosphatides; and, since the acid is known to be the parent acid of lecithin and kephalin, the authors propose to call it phosphatidic acid, and the Ca salt, calcium phosphatidate. The structure of the free acid is:



It differs from the acid of lecithin and kephalin in that these have both saturated fatty acids in equimolecular proportions, while phosphatidic acid is made up much more completely of unsaturated fatty acids, linolic and linolenic mainly, in ester linkage with the glycerol.

The phosphorus-free fraction, the 58 per cent not precipitated by acetone, was examined for fatty acids.[12] The material was saponified, and the fatty acids freed were identified as far as possible. The greater amount of these were found to be unsaturated acids, also linolic and linolenic probably. The saturated acids were mainly palmitic and stearic. The presence of oleic acid was not demonstrated, although it may be present in small amounts, and arachidonic acid is absent.

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[10] Chibnall, A. C., and Channon, H. J., II. Calcium salts of glyceridephosphoric acids. Biochem. Jour. 21:233–246. 1927.

[11] ———, IV. Further observations on diglyceridephosphoric acid. Biochem. Jour. 21:1112–1120. 1927.

[12] ———, III. The fatty acids. Biochem. Jour. 21:479–483. 1927.

Two non-phosphorus-containing compounds have been isolated from the phosphatide fraction, where they occur because of their similar insolubility in ether-acetone. These have been identified[13] as one of the higher paraffins, n-nonacosane, $C_{29}H_{60}$; and di-n-tetradecyl ketone, $C_{14}H_{29}\cdot CO\cdot C_{14}H_{29}$. These two substances constitute nearly half of the "phosphatide" fraction, and have previously been isolated only in small amounts in plant fats. The determination of the number of carbons in the molecule was done by X-ray analysis. The ketone was separated from the paraffin through the solubility of its ketoxime in a mixture of equal parts of light petroleum and acetone, a solvent in which the paraffin remains practically insoluble. The ketone was then regenerated, and found to have a melting point of 80.5–81° C. X-ray analysis again showed the ketone to contain twenty-nine carbons, and the CO group in the center of the chain. But because of the length of the carbon chain in the ketone, it was difficult to decide whether it was the symmetrical $CH_3\cdot(CH_2)_{13}\cdot CO\cdot(CH_2)_{13}\cdot CH_3$, or the unsymmetrical palmytyl-myristyl ketone, $CH_3\cdot(CH_2)_{14}\cdot CO\cdot(CH_2)_{12}\cdot CH_3$. Both of these compounds were then synthesized, and it was found that the latter has a different melting point (74–74.5° C.) from the naturally occurring ketone, while the symmetrical synthetic ketone had the same melting point as the one isolated from the cabbage.

In the summary and general conclusions[14] they bring out many interesting points in the physiological chemistry of plant fats. They also suggest that some term is needed to denote an ether extract, which is irrespective of the contents, and which does not connote any particular class of substances. They prefer that to BLOOR's term "lipide," which was proposed a few years ago.—C. A. SHULL.

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[13] CHIBNALL, A. C., and CHANNON, H. J., V. The isolation of n-nonacosane and di-n-tetradecyl ketone. Biochem. Jour. **23**:168–175. 1929.

[14] ———, VI. Summary and general conclusions. Biochem. Jour. **23**:176–184. 1929.

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STRUCTURE OF LARGE SOMATIC CHROMOSOMES

LESTER W. SHARP

(WITH PLATES XXII–XXIV AND ONE FIGURE)

Introduction

A number of years ago the writer published two papers (41, 42) dealing respectively with the large somatic chromosomes of *Vicia faba* and *Tradescantia virginiana*. According to the descriptions given therein, the chromosome, apparently homogeneous in the anaphase, became visibly differentiated during the telophase into more chromatic and less chromatic constituents by a process which has previously been called "alveolation." The less chromatic constituent mingled with other fluids to form the karyolymph, not necessarily persisting as a portion of the individualized chromosome throughout the mitotic cycle. The more chromatic constituent of each chromosome constituted a portion of the metabolic reticulum, assumed the form of a slender thread in the ensuing prophase, underwent a longitudinal division, and then thickened and shortened to become the double metaphase chromosome. This chromatic constituent was held to persist throughout the nuclear cycle, assuming the form of a slender thread (chromonema) only during the early prophase, when its longitudinal division appeared to take place.

Since that time certain other observers, notably **MARTENS** (23, 24), **KAUFMANN** (12–14), and **KUWADA** (16), have furnished cogent evidence in support of the view that in such chromosomes the two constituents mentioned are morphologically distinct and often visible, even in the metaphase and anaphase, and that the appearance of an alveolation in the telophase is due to a gradual decrease in the chromaticity of the less chromatic constituent, which renders increasingly visible a structure already present. The opinions of these observers differ as to the history of the less chromatic constituent, but the more chromatic constituent is thought to maintain the form of a more or less continuous chromonema throughout the mitotic cycle. KAUFMANN, moreover, following certain writers on animal chromosomes, contends that there are *two* chromonemata in the chromosome at all stages, owing to the early period at which the division of the more chromatic constituent is accomplished. In view of certain points to be brought out later, special mention should also be made of the statement of MARTENS that in *Paris quadrifolia* and *Listera ovata* the chromonema is single in the anaphase and the early prophase, and becomes doubled in the middle and late prophase, not by actual splitting, but by a less regular reapportionment of its substance to the two sides of the chromosome.

This general view, that the chromosome in its most condensed phases is essentially a matrix inclosing one or two chromonemata, has received strong support in the results of studies on chromosomes in the meiotic period. Especially fruitful have been the use of neutral violet extra on living chromosomes by KUWADA and SUGIMOTO (19) and KUWADA (17), the boiling water method of SAKAMURA (36, 37), the refinements of older methods by TAYLOR (46) and KAUFMANN (13), and the studies on the relation of hydrogen-ion concentration to visibility of chromosome structure by KUWADA and SAKAMURA (18) and SAKAMURA (36).

The theoretical value of such a conception of chromosome structure, which was developed especially by BONNEVIE (1–3) and VEJDOVSKÝ (47), has long been obvious in the light of other direct evidence that many chromosomes have characteristic morphological lengthwise differentiations, and the more indirect but scarcely less convincing evidence that they may have a lengthwise differentiation in function also.

In resuming this study of somatic chromosomes after an interval of several years, the writer has made use of certain special methods, to be described later, together with some of the methods used in the earlier investigations. The account of the chromosome cycle based on the results so far obtained is not intended to be complete, for a considerable number of significant points are still very obscure. The results are nevertheless sufficient to warrant a reinterpretation of the former observations, as well as a somewhat definite statement regarding the general course of events in the chromosome cycle as now conceived.[1] The data now at hand are in full harmony with the view that the more chromatic constituent of the somatic chromosome maintains the form of a chromonema, double in some cases at least, throughout the mitotic cycle, and is accompanied by the less chromatic constituent as a matrix at most if not all stages. The telophasic transformation does not involve an actual "alveolation" of the chromosome; hence this term should be abandoned.

Materials and methods

The present investigation was carried out on chromosomes in the root tips of *Trillium grandiflorum* (Michx.) Salisb., *Allium cepa* L., *Podophyllum peltatum* L., *Vicia faba* L., and *Tradescantia virginiana* L. Fixations were made in the usual manner with a number of fluids, including those of Benda, Flemming, Merkel, and Bouin. Particular attention was given to the action of Benda's fluid in order to obtain an adequate basis of comparison with the former results on *Vicia* (41), which had shown the superiority of this fluid for the fixation of the desired details. Moreover, the direct observations of MARTENS (24–27) on the action of this fluid under the microscope have indicated that it fixes the nuclei without producing any fundamental alteration in their visible structure. The most useful stain was iron-alum haematoxylin, both long and short procedures being employed. Among a variety of counterstains tried, eosin and chromotrope appeared to give the best differentiation of the chromosome matrix from the karyolymph.

Much of the uncertainty regarding the minute structure of chromosomes has been due to the difficulty of obtaining a suitable differentiation of the more chromatic and less chromatic constituents during late prophase, metaphase, and anaphase, when both constituents tend to stain deeply with haematoxylin or safranin. In the present study it was attempted to increase the difference in chromaticity between the two constituents, and thereby to gain a clearer picture of chromosome structure, by bringing about an incipient solution, digestion, or other alteration of the chromosome before fixation and staining, the thought being that the relative chromaticity of the constituents might be changed before their structural arrangement was noticeably disturbed. Such a procedure was suggested by the observations of OES (30, 31) on the effects of temperature, alcohol, toluol, and other reagents on the process of autolysis in plant and animal cells; and by those of NĔMEC (28, 29) on the solubility of chromosomes in hot water, with and without previous treatment with alcohol.[2] The "granular remains of mitosis" and the vague structures in treated chromosomes reported by these workers (NĔMEC 29, pl. IV) prompted testing the possibility of obtaining more definite and instructive results with such treatments.

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[1] The substance of this paper was originally presented before the General Section of the Botanical Society of America at its meeting in New York on December 28, 1928.

More than twenty different procedures were tried, chiefly on the root tips of *Allium*. These included the use of warm and hot water, ethyl alcohol of various strengths and temperatures, sodium phosphate, potassium nitrate, potassium hydroxide, sodium chloride, carbolic acid, corrosive sublimate, and other reagents. With very few exceptions these treatments yielded nothing of immediate value. Two of them, designated "method twenty" and "method four," proved to be more useful, and may therefore be described further.

METHOD TWENTY.—Fresh roots were placed in 12 per cent ethyl alcohol at 40° C. and allowed to remain for various lengths of time, after which they were fixed in Benda's fluid over night. They were imbedded in paraffin, sectioned, and stained in the usual manner. Anaphase chromosomes treated thus with warm alcohol for 30 minutes show only a slight swelling when compared with the controls, but they exhibit a decidedly clearer structure in properly stained preparations. After as long a treatment as four hours their appearance is much the same. Evidently the alteration which renders the chromosome matrix relatively less chromatic occurs within the first few minutes, after which any further change proceeds very slowly.

METHOD FOUR.—Fresh roots were placed in 95 per cent ethyl alcohol for 15 minutes and then transferred to distilled water at 90° C. for a few seconds or minutes. This treatment was followed by fixation in Benda's solution, as in method twenty. Roots which had been in the hot water for 15–60 seconds exhibited chromosome structure almost identical with that shown after the other method, whereas after four or five minutes the chromosomes appeared translucent with no clear structure. This method was suggested by NĚMEC's (28) observation that previous treatment with alcohol retards the rate of solution in hot water. Without such preliminary treatment, water at any temperature above 60° C. swells and dissolves the chromosome too quickly to be of much service. The statements of NĚMEC and OES are confirmed that "resting" nuclei are far more resistant to the drastic action of hot water than are the metaphase or anaphase chromosomes.

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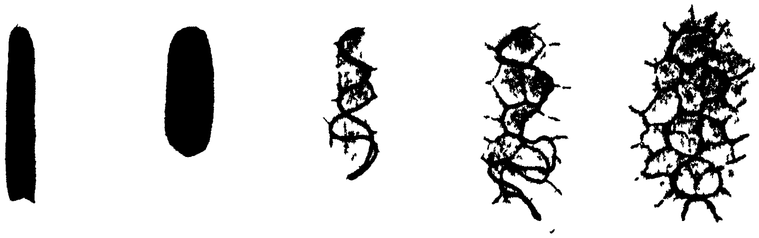
[2] See also the general accounts of ZACHARIAS (48) and PRATJE (33).

This application of such methods can scarcely be regarded as more than a preliminary survey, since it is probable that any of them which show promise could be considerably refined by giving special attention to such factors as, for example, the conditions under which the roots are grown and the pH value of all the solutions employed. As will be pointed out in the description of the anaphase, it seems abundantly evident that the structure described is not produced, but only rendered more visible, by the special treatments, since it frequently appears with marked clearness in chromosomes not so treated.

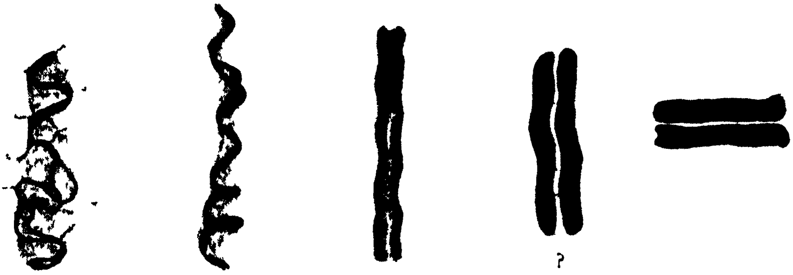
Preliminary statement

A brief summary and diagram of the interpretation of the somatic chromosome cycle are first presented, in order to facilitate detailed description and to furnish a convenient basis for any future discussion. The scheme here outlined should not be regarded as one to which all the species dealt with conform rigidly in all particulars; especially should it not be applied without further investigation to chromosomes in tissues of other types. It is an interpretation which seems to represent a somewhat close approach to the state of affairs in all the large somatic chromosomes here studied, but only future researches can show in what particulars it is inadequate, or how it must be modified to conform to the conditions found in other chromosomes.

In the anaphase the chromosome is made up of a matrix and a more chromatic constituent, the latter having the form of a crooked thread or chromonema (text fig. 1). The thread often appears double, that is, there are two chromonemata, which may either remain very close together (upper part of chromosome) or separate rather widely (lower part), both now and in the other phases of mitosis. At the end of the anaphase (tassement polaire) the chromosome shortens.



Anaphase Tas pol. Telophase Interphase Metabolic stage



Early prophase "Spiral" stage Medium prophase Late prophase Metaphase

FIG. 1.—Diagram of successive alterations undergone by large chromosome during course of one mitotic cycle: chromosome matrix shown in gray and chromonemata in black, exact stage of prophase at which each of the two chromonemata becomes doubled is undetermined.

In the telophase the chromaticity of the matrix decreases, leaving the chromonemata more plainly visible. These are more contorted than in the anaphase, owing to the shortening of the chromosome as a whole. They become connected with the chromonemata of the neighboring chromosomes by anastomoses to form a reticulum.

The telophasic alterations continue into the interphase, when the individual chromonemata can still be traced in favorable preparations. Rapidly multiplying nuclei may pass into the next prophase from this stage. When the divisions are less rapid the telophasic alterations are carried further, the anastomoses and the main portions of the chromonemata becoming more and more alike, so that the latter are no longer discernible in the fine-meshed metabolic reticulum. The chromatic matter is not resolved into visible granules. Meanwhile the matrix substance of the several chromosomes becomes entirely achromatic and apparently continues. The precise relation of this substance to the karyolymph in which the reticulum is imbedded is undetermined.

In the early prophase the reticulum is resolved into separate tracts representing the chromosomes, in each of which the two chromonemata become increasingly visible as the anastomoses disappear. The matrix of each chromosome becomes distinguishable from the surrounding karyolymph. The chromonemata become increasingly distinct and regular, giving the aspect characteristic of the spiral stage.

In the middle portion of the prophase the chromonemata straighten, thicken, and diverge somewhat as they take up positions in the two borders of the flattening matrix. The matrix becomes more chromatic, and probably begins its division.

In the late prophase the chromosome matrix is shorter, thicker, and still more chromatic, and its division is completed. The chromonemata are again contorted, but owing in part to the chromaticity of the matrix, their exact structure is obscure. In view of their evident duality in each half-chromosome in the metaphase so soon to follow, together with certain very suggestive appearances in the late prophase, it is highly probable that each chromonema undergoes division some time during the latter portion of the prophase, as indicated provisionally in the diagram. The two chromonemata thus formed are to separate finally in the second succeeding anaphase.

In the metaphase each half-chromosome (daughter chromosome) is a matrix containing two chromonemata, so that the entire metaphase chromosome is double with respect to the matrix and quadruple with respect to the more chromatic constituent. The intimacy of association of the two chromonemata in each half-chromosome varies greatly, making the duality obvious in some cases but not evident in others.

Observations

The following description does not present a continuous account of the alterations undergone throughout the mitotic cycle by the chromosome in a given species; rather it takes up the phases of mitosis in turn, describing whatever chromosomes have yielded information, regardless of species. There are still gaps or points of uncertainty in the known history of the chromosome in each species, but the measure of general agreement between all the species warrants the adoption of the provisional scheme presented in the foregoing pages, and lends weight to the opinion that actual differences are chiefly those of degree.

The anaphase is considered first because of the obvious advantage of beginning with the stage at which the chromosome is most clearly evident as an individual. Furthermore, the special technical methods were employed with particular reference to their effect upon metaphase and anaphase chromosomes, in which the structure is ordinarily obscured by the chromaticity of the matrix.

For purposes of comparison it should be noted that all of the figures in the plates are drawn at the same magnification, with the exception of figs. 1, 8, 17, 18, 20, 26, 39, and 49, which show only half as much enlargement. These smaller scale drawings show the general appearance of the nuclei and facilitate identification of the various phases.

ANAPHASE

In most ordinary preparations the anaphase chromosomes appear as uniformly black or gray rods (fig. 1, *Vicia*). That such chromosomes are in some degree double is strongly suggested by the notch and light median line occasionally seen at the distal end (fig. 2), as well as by the moniliform outline frequently reported (KAUFMANN 13). The most extreme condition so far observed is in *Trillium* (fig. 3), in which the anaphasic doubleness may be as conspicuous after fixation in Benda's fluid as one expects it to be in the late prophase or the metaphase after some other fixations. These chromosomes are somewhat flattened, and have deeply chromatic borders separated by a more readily destaining median region. The light region is plainly not the familiar median line often produced by refraction.

An apparently different type of structure is revealed in anaphase chromosomes by special methods, and frequently by ordinary methods as well. In fig. 4 are shown two chromosomes and a portion of a third in a root tip of *Vicia* fixed in Benda's solution and stained with iron-alum haematoxylin (short procedure). Here the chromatic constituent has the form of a very evident zigzag or spirally coiled chromonema running more or less continuously throughout the length of the chromosome. At the upper end of each large chromosome there is a suggestion of duality, while in the small portion of a chromosome there appear to be two chromonemata, although this is not a particularly clear case. Such internal chromatic tracts in chromosomes fixed in Flemming's weaker solution were figured in an earlier paper on *Vicia* (41), but the proper significance was not assigned to them.

The same type of internal structure appears clearly in chromosomes of *Allium* prepared by method twenty. Except for the decreasing length and increasing thickness of the chromosome, the appearance is the same in early anaphase (fig. 5), medium anaphase (fig. 6), and the tassement polaire stage (fig. 7). The matrix has a somewhat even outline, and is distinctly delimited from the surrounding fluid. In some regions the more chromatic element exists as an apparently single zigzag or spiral thread, whereas in other regions there are as clearly two of these. The probable key to this situation is found in cases such as that in fig. 6. In *a* there is but one evident chromonema; in *b* the chromonema is double at the distal (lower) end; in *c* there are two widely separated chromonemata, as in fig. 5*bc*. Later a similar series of aspects will be reported for the telophase and the prophase.

A useful hypothesis covering these phenomena in such chromosomes is that the chromonematic element is actually double at all stages, and that its halves vary greatly in their spatial arrangement, owing in part to the conditions of growth, methods of fixation, and other unknown local conditions. Moreover, much variation in appearance depends upon the angle from which the associated chromonemata are viewed. Consequently, the chromosome may appear to have a single chromonema, a double chromonema, or two chromonemata. To what extent fixation may effect a divergence of the threads, or their closer approximation as suggested by KAUFMANN, must remain for the future to determine. That such spiral structures are artifacts of fixation is scarcely a plausible view, now that KUWADA and SUGIMOTO (19) have demonstrated them in fresh meiotic chromosomes with neutral violet extra, and since SAKAMURA (36, 37) has dissolved away the matrix, leaving the spirals intact.[3]

TELOPHASE

In the comparatively young telophase nucleus (fig. 8, *Vicia*) the individual chromosomes stand out conspicuously, owing to their straightness and the transparency of the non-chromonematic substances. Their exact structure, however, is not so easily distinguished as would appear from that fact. The chromonemata undergo considerable distortion, especially at places where anastomoses connect them with one another; also, the chromosomes may soon begin to curve somewhat, and the spaces between them gradually become less evident.

The middle chromosome in fig. 8 is shown on a larger scale in fig. 9. Although the precise arrangement of the chromatic substance is uncertain at a number of points, it is plain that it continues to be in the chromonematic form seen in earlier stages. In view of what has preceded, it is best interpreted as a pair of chromonemata. In the left-hand chromosomes in figs. 10 and 11 the two chromonemata are more obvious, since they tend to run more nearly parallel in certain regions. In fig. 12 (*Tradescantia*) they appear to be widely separated above and more closely associated below, although this apparent difference may be due entirely to the angle of view. It is to be doubted whether the two chromonemata in such chromosomes as that shown in fig. 12 are so interlaced or intertwined that they could not be moved apart laterally without becoming entangled. This point will be mentioned again in the account of the prophase.

In figs. 13 (*Tradescantia*) and 14 (*Allium*), which are drawn from nuclei a little more advanced than that of fig. 12, there appears to be but one chromonema. Such an aspect is undoubtedly due in some cases to the fact that two chromonemata lie in the same line of vision, or to imperfect fixation or staining. I am of the opinion, however, that in the telophase, as in the anaphase, the two chromonemata vary considerably in the intimacy of their association, so that they may at times lie in contact and form what appears to be a single thread. It is even possible that this tends more strongly to occur as the telophase advances, either naturally or because of imposed conditions. This is suggested by the fact that in these preparations the appearance in the succeeding interphase is so often one of singleness (figs. 15, 16).

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[3] While this paper was in press, the visibility of the chromonema in the chromosomes of living *Tradescantia* sporocytes in Ringer's solution was reported by MARTENS (Bull. Acad. Roy. Belg. Cl. Sci., Ser. V 15: 160–169. 1929).

Those who have studied such nuclei are aware of the basis for the interpretation of the conditions shown in figs. 8–14 as results of alveolation, especially when it is borne in mind that in very young telophase nuclei (between the stages of figs. 7 and 8) the chromosome matrix is more chromatic and tends to obscure the structure actually present in most ordinary preparations. In such preparations the first indication of a loss of chromaticity in the matrix is the appearance of translucent spots, and as the process continues these spots become the spaces between and around the chromonemata. Thus they may appear to originate in very irregular positions within each chromosome, as stated in earlier papers (41, 42). The situation is well illustrated in KAUFMANN's figures of meiotic chromosomes (13, figs. 56–63). Now that we know the chromonemata to be present even in the preceding anaphase, it is evident that the structure observed within telophase chromosomes is not actually produced at this time by alveolation or any other process, but is simply rendered visible through a reduction in the chromaticity of one of the constituents of the chromosome. The only conspicuous new elements are the anastomoses, which join the chromonemata of neighboring chromosomes to form the interphasic reticulum.

Brief reference may be made here to the views of certain earlier writers with respect to telophasic doubleness. It was the opinion of LUNDEGÅRDH (20, 21), FRASER and SNELL (9), DIGBY (6, 7), FRASER (8), and others that median alveoles appear in telophase chromosomes and develop into a more or less complete longitudinal split, which persists through the succeeding interphase and prophase. Accordingly the chromosomes of the telophase were figured as somewhat ladder-like structures, the two side pieces corresponding to the future daughter chromosomes. This particular interpretation of the telophase was opposed in my earlier papers, since it was found (1) that the alveoles appeared not only in a median position but along the periphery as well; (2) that the chromosome at this time was still a cylinder and not a flattened ladder; and (3) that the side pieces of the ladder-like telophase chromosome did not develop directly into the longitudinal halves of the succeeding middle prophase chromosome, but gave rise in the very early prophase to an apparently single slender thread incorporating also the cross pieces, this thread then splitting to give the duality of the middle prophase. It now appears that the foregoing investigators were correct in interpreting some (although not all) of the telophasic aspects they observed as chromosome doubleness, although they shared my error in supposing the telophasic alteration to consist in an actual alveolation. Furthermore, they were wrong in believing the splitting to take place in the telophase as the result of such a process. Later it will be shown that splitting is in all probability prophasic, as I (41, 42), KUWADA (15), OVERTON (32), and others have contended, although it does not occur in the manner hitherto supposed.

MARTENS (23, 24) also holds that the partisans of the theory of splitting in the telophase were correct in their general observation of chromatic duality at that stage, but were wrong in their interpretation. For him, however, the duality they observed does not represent an actual definitive splitting of the chromosome as they contended, but results from the temporary accumulation of the chromatic material along two lines corresponding to the two edges of a troughlike structure formed by this material at earlier stages. This interpretation he supports by the statement that an unsplit chromonema is present in the early stages of the succeeding prophase. The "trough" conception does not seem applicable to the appearances presented by my preparations; moreover, it will be pointed out later that the prophasic chromonema is double from its earliest stages. Hence it can only be concluded that, although the investigators referred to were wrong in their general interpretation of the telophase, the duality they observed at that stage was at least in some cases (cases in which the chromonemata were widely separated, as in my fig. 12) actually that which develops into the doubleness of the succeeding middle prophase.

INTERPHASE

As the telophase advances and passes into the interphase, the nucleus increases in size and the reticulum formed by the anastomosed chromonemata tends to become more nearly uniform throughout. The individual chromosomes accordingly stand out less clearly, while the analysis of their structure becomes increasingly difficult. A small piece of an exceptionally clear interphasic reticulum of *Vicia* is shown in fig. 15. Here the contorted chromonemata appear to be single and rather thick, as if the two in each chromosome had become very closely associated, as suggested in a previous paragraph. Somewhat the same appearance is presented by a treated interphase nucleus in *Allium* (fig. 16). Other cases are better interpreted as two more loosely associated chromonemata, the aspect of each chromosomal tract being more like fig. 12. Hence it seems that the several conditions present at earlier stages owing to a variation in the mutual behavior of the chromonemata may be continued into the interphase.

The foregoing interpretation of the development of the interphasic reticulum from the chromonemata of the anaphase is in its essential features similar to that of KAUFMANN (13). The most noticeable difference is with respect to the duality visible in the later stages: I do not find in my preparations such regularly intertwined chromonemata throughout the telophase nucleus as KAUFMANN shows in his figs. 24, 27, and 29. With respect to the interphase, my fig. 15 and his fig. 32 are in closer agreement except for the degree of angularity of the meshes of the reticulum. In his figure many of the zigzag threads appear to be single, although duality is visible in certain places, as I have also observed. It is my intention to study more intensively the telophasic alterations in the chromosomes of *Trillium*, for in view of their well developed duality in the anaphase it would seem that they should yield valuable evidence as to the constitution of the interphase nucleus.

One of the most puzzling problems pertaining to the telophase and interphase is that of the fate of the chromosome matrix, and hence the constitution of the achromatic substance in which the reticulum is imbedded. A discussion of the various possibilities has been presented elsewhere (SHARP 43) and need not be repeated here. In a young telophase nucleus of the stage shown in fig. 8 the chromosome matrix appears to be distinct from the fluid which occupies the spaces between the chromosomes, and which is probably entering the now enlarging nucleus. Later on, however, when the reticulum becomes more uniform and the matrix less chromatic this distinction is not evident, and I have inclined to the opinion that the chromosome matrix and the fluid entering the nucleus during the telophase intermingle to form the transparent karyolymph of the interphase and metabolic stage. This is also the view of KAUFMANN; MARTENS, on the contrary, believes the two substances to remain distinct. As yet no published figures can be regarded as decisive on this point.

The general appearance of an advanced interphase (of *Tradescantia*) is shown in fig. 17. There are no obvious boundaries between the chromosomes at this stage, although in properly oriented nuclei one may still distinguish chromonematic tracts representing the chromosomes. The extent to which the telophasic transformation may be carried varies with the interval between successive mitoses. In any given case it may be impossible to be sure that the nucleus is to enter the next prophase without further alteration toward a metabolic condition, but the frequency with which the various stages occur in some sections, and comparisons of their arrangements in certain cases, indicate that the turning point may be reached before the stage of fig. 17, that is, while the chromonematic elements of the individual chromosomes are still distinguishable. In rapidly multiplying nuclei, conditions more advanced than that of fig. 17 are comparatively rare, but as the interval between mitoses becomes longer, completely developed reticula are found.

METABOLIC STAGE

A fully formed metabolic reticulum from the same section as the interphase of fig. 17 is shown in fig. 18. The structure of this nucleus is similar to that of the preceding stage in all respects save the greater tenuity of the chromatic strands and the smaller size of the meshes in the reticulum. Accordingly, tracts representing individual chromosomes can no longer be identified. The network is uniform throughout, and lies in the transparent karyolymph which affords no evidence of the presence of two physically distinct constituents. When viewed under low magnification or when insufficiently stained, the nucleus may seem to be filled with discrete granules, but careful observation of good preparations always reveals the presence of strands connecting the granules. In other words, the supposed granules are only those regions of the chromatic strands which are thicker or are being viewed endwise.

The validity of interpretations of nuclear structure based on fixed material alone may rightly be questioned. The usual statement of those who have studied living cells under carefully controlled conditions is that most metabolic nuclei appear faintly granular or entirely homogeneous except for the nucleolus. SCHAEDE (39) found the living nuclei of young *Allium* and *Vicia* root cells to be homogeneous in appearance, not showing the reticula reported by LUNDEGÅRDH (20, 21) until they become moribund. In older root cells and in the stamen hair of *Tradescantia* he observed a granular appearance. His general conclusion is that the chromatic matter in metabolic nuclei has the form of discrete granules suspended in the karyolymph, these two constituents not being visible in some nuclei because of their similarity in refractive index. The reticulum he regards as an artifact due to degenerative changes in living cells or to fixation in ordinary preparations.

MARTENS (24–27), working with the roots of *Listera* and other plants, the leaf of *Elodea*, the stamen hair of *Tradescantia*, and especially the plumose stigmas of *Arrhenatherum elatius* and other grasses, showed that under carefully controlled conditions of observation unharmed nuclei may reveal the presence of reticula, and not merely discrete granules. Furthermore, he watched the action of Benda's and Bouin's fluids upon such nuclei under the microscope, and found that the reticular structure visible before fixation remained essentially unchanged, although there might be a slight swelling of the filaments, especially at the nodes of the reticulum, and an increase in their visibility through alterations in refractive index. The same details appeared in material fixed for longer periods and stained with iron-alum haematoxylin in the usual way. SCARTH (38) also reported "an irregular framework of soft gel" in the living nuclei of *Tradescantia*, a finely granular aspect in *Elodea*, and apparent homogeneity in *Spirogyra* and the fruit of *Symphoricarpos*. The visibility of the reticulum in these cases is said to vary inversely with the pH value and more or less directly with the viscosity of the nucleus as a whole.

The opinion which is best justified by a comparison of all reported observations is that the chromatic matter in metabolic nuclei of the type here dealt with exists in the form of a reticulum composed of anastomosed chromonemata. The actual appearance of the living nucleus (whether homogeneous, granular, or reticulate) depends upon the refractive indices of the chromatic matter and the karyolymph. Increasing divergence of these indices through natural or artificial causes brings into view first the nodes ("granules") of the reticulum and then the finer connecting strands, in much the same manner that progressively deeper staining does so in fixed material. If we regard as valid SCHAEDE's argument that the appearance of granules in the *Tradescantia* stamen hair nucleus as opposed to the homogeneity of the root nuclei he saw is due to different refractive properties in different unharmed nuclei, it would seem to be equally applicable to the connecting filaments observed by MARTENS in nuclei of the same kind; in fact, as MARTENS points out, SCHAEDE's own photomicrograph (pl. V, fig. 6*f*) shows crooked filaments and not only granules.

It is possible, of course, that there are other types of nucleus to which the foregoing interpretation will not apply. GROSS (11) reports brownian movement of apparently isolated granules in the nuclei of the salivary gland of *Limnaea*, and SHIWAGO (44) describes filaments which seem to be unconnected with one another in frog leucocytes. The observations of CHAMBERS (4) on the prophase stages in living spermatocytes of *Dissosteira* afford strong support to the view that fixation does not produce the structures in question, but only renders them more visible. That actual coagulation artifacts do occur at times goes without saying.

PROPHASE

EARLY PROPHASE.—For convenience of treatment the whole prophase may be subdivided into three portions. What is here called the early prophase is characterized by the resolution of the reticulum into its individual chromosomes, in each of which the chromatic constituent appears as two associated chromonemata. The changes undergone by the chromosomes are in many respects the reverse of those in the telophase, at least so far as appearances are concerned; if it were not for the early disappearance of the anastomoses, the resemblance between telophase and prophase nuclei would be even closer.

As the prophase is initiated, the first visible change consists in the attenuation of the anastomoses along certain lines in the reticulum (fig. 19). This alteration may begin in a metabolic reticulum (fig. 18) or in the relatively coarser interphase stage (figs. 15, 17). The successive cells in a row in the root do not often show a regular succession of mitotic phases; this, together with the resemblance of late telophase and early prophase nuclei, often renders it impossible to be certain regarding the immediate past of a given nucleus in these stages. Somewhat later the prophase nucleus is easily distinguishable from the earlier telophase nucleus (compare figs. 20 and 26 with fig. 8) by its larger size and more evenly rounded contour, the relative scarcity of anastomoses, and the absence of a similar sister nucleus in the same or adjacent sections. In the very early prophase nucleus, in which the reticulum is just beginning to break up, the chromatic tracts (chromosomes) tend to be broader and the anastomoses more tenuous than in the early interphase (compare figs. 19 and 15, drawn from nuclei in the same preparation). As the actual turning point between successive mitoses is approached, all these criteria naturally become less useful, since at this point the differences vanish. However, they do permit one to follow the telophase far enough along, and to trace the prophase far enough backward, to make it practically certain that in regions of the root where the nuclei are multiplying rapidly, and where very advanced interphases are relatively rare, the turning point may be reached before the constituent chromonemata become indistinguishable. Accordingly, the stage shown in fig. 19 may be considered to follow that of fig. 15 directly. The probability that this interpretation is valid is very high, although more convincing proof should be sought in some tissue showing a regularly arranged series of mitotic phases.

The exact structure of the chromatic tract representing the chromosome at the beginning of the prophase is difficult to determine. In some cases there appear to be two chromonemata more or less separated, but in the majority of clear examples observed the chromatic thread appears to be for the most part single. It is partly for this reason that these examples are more clearly analyzable. In the lower part of the left-hand chromosome of fig. 19 a single zigzag seems to be coming into prominence; that the lighter strands represent a second chromonema is doubtful. In view of the tendency of the two chromonemata to associate closely in the telophase and the interphase, and their obvious closeness in the prophase to follow, it seems more than likely that such apparently single zigzags as that in fig. 19 consist of two parallel elements.

As the anastomoses disappear and the chromatic tracts become isolated, the nucleus has the appearance shown in fig. 20 (*Podophyllum*). Under moderate magnification, as in this figure, most of the chromatic matter in each tract appears to form a single crooked thread with duality suggested at some points. This is the "apparently single slender thread incorporating also the cross pieces" of the telophase chromosome referred to earlier in the paper. Under high magnification the duality can be seen plainly. Several chromosomes or portions of chromosomes from nuclei of *Vicia* in this stage are illustrated in figs. 21–23. In the lower portions of the chromosomes in fig. 21 the chromonemata lie closely parallel, while in the upper portion of fig. 21*a* they seem to be widely separated. The latter condition is rather plain in the upper portion of fig. 22*b*. Some chromosomes, such as those illustrated in fig. 23, are scarcely interpretable in most regions: they are much like the "alveolized" prophase chromosomes figured in my earlier papers. The fact that the chromosomes of figs. 22 and 23 are from the same nucleus indicates that the "alveolized" examples are only poorer pictures of the chromonematic structure shown to better advantage in the other figures. A similar variation is found in *Podophyllum:* in fig. 24 the general structure appears to be very irregular and the chromonema single at most points, but in another chromosome from the same preparation (fig. 25) the chromonema is clearly double at the end where the matrix has been destained to just the right degree. All of these cases point to the conclusion that two chromonemata are present in each chromosome from the inception of the prophase, a point of the greatest importance with reference to any interpretation of the chromosome cycle.

As a result of the disappearance of anastomoses and the increasing regularity in the arrangement of the chromatic matter, the conspicuous "spiral stage" of the early prophase is reached (fig. 26, *Podophyllum*). The ease with which this stage is recognized is due in part to the tendency of the two associated chromonemata in each chromosome to lie very close together, whatever may have been their previous position. Figs. 27-38 show portions of chromosomes in this stage more highly magnified. Here, as in the still earlier prophase, the chromatic thread may appear single or double, depending upon fixation and other circumstances In *Vicia*, for example, it appears single in fig. 27 (Flemming fixation) but plainly double in fig. 37 (Benda). In *Trillium* it may be apparently single in most regions (fig. 29) or obviously double (fig. 30) in the same preparation (Benda). In fig. 31 (*Podophyllum*) are shown three chromosomes in the same nucleus: in the uppermost the successive coils of the spiral thread are very close together; in the middle chromosome the thread has for some reason straightened out in a curious manner, but shows no duality; in the lowermost the thread is unmistakably double in its middle region, where the extended condition makes it certain that the double appearance cannot be due simply to a closeness of successive coils. Duality of the thread is further shown in *Tradescantia* (figs. 32-35), although the appearance varies here as in the foregoing examples.

A particularly clear example is shown in fig. 37 (*Vicia*). Here the two chromonemata have thickened a little more than is usual before the mid-prophasic straightening, which makes it possible to follow the course of the threads with less uncertainty. In this portion of the chromosome they form a strikingly regular spiral. Moreover, careful examination of the points at which the two threads cross each other strongly suggests that they are not simply intertwined, but that their relative position is such as would permit them to move apart to two sides of the enveloping matrix without becoming entangled. That they should be able to do this is suggested further by the fact that they have been brought into the spiral form by the shortening of the matrix at another stage of the mitotic cycle, as will soon be shown. KUWADA (17) has considered this aspect of the problem, and has discussed it instructively with the aid of wire models. He points out that if two wires forming a common spiral are twisted once around each other for every turn of the spiral, but in the direction opposite thereto, they will come apart without entangling. In fig. 37 it appears very much as if the chromonemata might diverge, one toward and one away from the observer, without becoming entangled; it is scarcely possible, however, to be altogether certain of this. Later on in the prophase they do become well separated and parallel, but to what extent this may involve twisting and other modes of rearrangement is not known.

Attention may be directed again to the variation in the appearance of the chromatic matter, especially in different preparations and lots of material, and to the idea that this is due in considerable measure to influences causing approach or divergence of the two associated chromonemata during growth and fixation. It is well known that the distinctness of half-chromosomes in the metaphase varies in this way, and it therefore seems not unlikely that corresponding effects are produced at other stages also.

This variable appearance in the slender chromatic threads of the prophase (singleness in one region and doubleness in another) has usually been interpreted as an actual splitting at this stage, particularly when the threads have undergone some straightening before thickening (fig. 38). Such was the interpretation given in the paper on *Vicia* (41), fig. 10 of that paper showing about the same stage as fig. 38 of the present one. That such an interpretation is valid for the chromosomes in question can no longer be maintained, in view of the demonstrated duality at earlier stages. In many preparations an apparently single crooked thread emerges in the early prophase and becomes double later, but in other preparations of the same lot of material the early thread also shows the duality.

This point is of further interest in connection with the problem of the meiotic prophase. To cite briefly but one recent investigation, SZAKIEN (45) reports that in *Osmunda* the thread arising from each chromosomal tract in the presynaptic stages is single, and that no duality is visible in such threads until well after their parasynaptic association has been accomplished. Although phenomena in somatic nuclei must be used with some reserve in explaining the meiotic changes, the present observations tend to support the suspicions of many cytologists that the chromosomes in meiosis are in some measure constitutionally double some time before their synaptic association begins.

The chromosome matrix is not shown clearly in most of my figures of the early prophase, largely because they were drawn from preparations destained to show the chromonemata as clearly as possible. Moreover, in the earliest stages it has only the slightest affinity for the haematoxylin stain. In darker or counterstained preparations the matrix can be seen, and appears to be distinct from the karyolymph (figs. 23, 25, 27); but later on, when the slender chromatic threads straighten (fig. 38) it becomes difficult or impossible to identify it in many regions. I incline to the view of MARTENS that the matrix exists throughout this stage as a thin layer about the chromatic threads and as small accumulations held in their curves. The alternative view is that of KAUFMANN, who believes the matrix substance to differentiate anew in the later prophase, only the chromonemata preserving the identity of the chromosomes through the interphase and early prophase. As already emphasized, the whole subject of the less chromatic component of the chromosome and its relation to the karyolymph deserves further intensive study.

MIDDLE PROPHASE.—The change characterizing the middle portion of the prophase is the straightening and thickening of the chromonemata; this gives the familiar mid-prophase aspect shown in fig. 39 (*Podophyllum*). In what measure this change involves an actual elongation of the chromosome as a whole is undetermined. In fig. 38 the straightening threads are still slender, which would seem to necessitate a considerable increase in length; whereas, in fig. 37 a noticeable amount of thickening has occurred while the threads are still contorted. The aspect varies to some degree with fixation, but it nevertheless seems likely that the time relation of straightening and thickening is not always precisely the same. Were these changes to proceed at the proper relative rate, there would be no elongation of the chromosome as a whole.

Although the chromonemata in any given region of a chromosome have become much straighter, they remain more or less twisted about each other, and together follow an irregular course through the nucleus (fig. 39). The free ends of chromosomes not at either surface of the section show that there is no continuous spireme. The associated chromonemata tend to diverge more than in the earlier stages of the prophase, and occupy the edges of the now flattening and broadening matrix. A rather extreme case is shown in fig. 40. In the light preparations from which this and a number of the accompanying figures were drawn, the matrix is colorless, but the delicate strands connecting the chromonemata can be distinguished. In fig. 41 is a small portion of a chromosome in which the two chromonemata at first appear to be twisted, but in which careful focusing seems to show the same thread above the other at all crossing points. Fig. 42 illustrates the lumpy appearance so often presented by the threads at this stage. Nothing new can be contributed here regarding the origin and significance of these lumps, or chromomeres.

The prophasic changes so far described are of special interest in connection with the view of MARTENS (23, 24) that the chromonematic element of the chromosome does not actually split, but that its substance undergoes a less regular reapportionment or redistribution ("répartition bilatérale chromatique") to the two borders of the flattening matrix. According to this view, the two chromatic strands in the middle prophase (fig. 42) and later are the result of such a process initiated in a single thread (chromonema) present in the earlier prophase. Since KAUFMANN and I have now shown that there are two chromonemata in the chromosome (that is, that the chromatic thread is not single but double) from the beginning of the prophase, it follows that I (41) was in error in attributing the doubleness of the middle prophase to a splitting in the early prophase, and also that the interpretation of MARTENS is equally untenable. As the two chromonemata gradually straighten and take up positions at opposite edges of the matrix, they with their connecting strands often present aspects similar to those figured by MARTENS; but the two threads supposed by him to be formed in the middle and late prophase are actually present much earlier, so that another explanation of their origin must be found.

The probability that the morphological division of the chromatic substance is initiated in the middle prophase (in each of the two chromonemata present from an earlier stage) will be discussed later.

LATE PROPHASE.—The late prophase is characterized by a thickening and shortening of the chromosome with the resultant contortion of the chromonemata and division of the matrix. There is also evidence that the doubleness of each chromonema becomes visible at this time; if so, this constitutes the most important feature of all.

The effect upon the appearance of the chromosome produced by its shortening and thickening, and by the contortion of the chromonemata, is illustrated in figs. 43–47, only some of which show the matrix. These five figures represent respectively chromosomes of *Allium* prepared by method twenty, *Vicia* fixed in Flemming's strong fluid, *Podophyllum* fixed in Benda's fluid, *Tradescantia* fixed in Flemming's fluid, and *Trillium* fixed in Benda's fluid. All are from preparations stained with iron-alum haematoxylin; that of *Trillium* was counterstained with eosin and shows the matrix more plainly. Just as it is difficult to say in what measure the straightening of the chromonemata in the middle prophase is correlated with an elongation of the chromosome as a whole, so in the late prophase one cannot be sure of the degree of interdependence of chromosome shortening and chromonema contortion. That the two changes are causally related, however, can scarcely be doubted. Whether the chromonemata themselves undergo any actual changes in length with their alteration in form during the mitotic cycle is not known; it is even conceivable that they maintain a nearly constant length, and vary in appearance from stage to stage according to the shapes assumed by the matrix in which they are confined.

As the prophase advances the chromosome matrix gradually increases in chromaticity, so that the internal structure of the chromosome tends to become obscured. As in the anaphase, the chromaticity may be decreased by special treatment; moreover, it is sometimes sufficiently less in material not so treated to reveal the structure rather clearly. In fig. 46 the two chromonemata are rather well separated in the matrix. They appear at first glance to be twisted; but one lies above the other, and if this chromosome could be viewed from the side the appearance would probably approach that of fig. 47*a*, in which the chromosome ribbon lies flat in the section.

The exact stage at which the matrix becomes completely divided is not easily determined; observations on this point are insufficient to warrant very positive statements. In the very late prophase chromosomes in fig. 47 the matrix appears to be continuous from one side to the other; whereas, in the chromosome in fig. 48, which was observed in a nucleus apparently of the same age, the matrix has divided. Doubtless the inception of the division in some regions may occur at a considerably earlier stage: this is suggested by the unusually wide divergence of the two chromonemata sometimes observed (fig. 40). How much variation there may be in this respect is not known.

At the end of the prophase, therefore, the two daughter chromosomes which are to separate in the following anaphase are defined, their matrices having become distinct only recently, but their chromonematic elements having been distinct for a much longer period. Before proceeding with a description of subsequent phases, special consideration must be given to the chromonemata.

It has already been stated that two chromonemata are present in the anaphase chromosome, and it will soon be pointed out that this chromatic duality is evident in each half of the double metaphase chromosome, at least in some cases. It would therefore seem that a subdivision of each of the two chromonemata seen in the early prophase must occur some time before the metaphase. Direct and convincing evidence on this point is not easily obtained. In the late prophase chromosomes shown in figs. 44, 45, and 47 there are suggestions of doubleness at certain points (indicated by arrows), but the real significance of these aspects cannot be evaluated safely at present. It is for this reason that a mark of interrogation appears at the late prophase stage in the diagram in the text. This is essentially the point to which KAUFMANN (13) carried his study of the chromonemata in *Tradescantia pilosa:* his figs. 47, 48, and 50 show strong hints of chromatic duality in each half of the late prophase chromosome. On theoretical grounds we could expect the division to be initiated while the chromonemata are relatively slender, as in the middle prophase, even though it might not attain visibility until the chromosome had thickened somewhat in the late prophase. It is intended to investigate this matter further, especially in the large chromosomes of *Trillium*.

The process by which the chromonema becomes doubled longitudinally is as obscure as ever, perhaps more so. In view of the actual division of other protoplasmic units, such as cells and plastids, it is natural to assume that the chromonema also is doubled by an actual fission. To what degree this is the case, that is, to what extent and in what manner the molecular and colloidal constituents of the chromonema are involved in division, can only be conjectured. It is conceivable that a process similar to that supposed by MARTENS to occur in a single thread in the prophase may take place in each of the halves of this actually double thread in the preceding prophase, but at present there is no reason to regard this as very probable. Since such great theoretical importance attaches to this point in the mitotic cycle, it is to be hoped that a way will be found to obtain more decisive observational data.

If the interpretation of the course of somatic nuclear division embodied in the diagram is correct, the chromosome as a whole does not become fully divided until the fission of the matrix late in the prophase of the mitosis in which the resulting daughter chromosomes are to separate; whereas, its more chromatic constituent (chromonema) has been divided since the prophase of the preceding mitosis. If, as some observers maintain, the matrix does not remain distinct from that of the other chromosomes and from the karyolymph during the interphase, it would be necessary to say that the double "chromosome" (chromonema) of the early prophase becomes surrounded by a new individual mass of matrix substance, making a single "chromosome" (matrix) which is divided in the late prophase. The conception of the autonomy and genetic continuity of the chromosome would accordingly apply only to the chromonematic element. To what extent the less chromatic constituent may participate in determining the morphological and functional peculiarities of the chromosome is an interesting and important question.

METAPHASE

An illustration of the stage transitional from the late prophase to the metaphase is introduced (fig. 49, *Podophyllum*), not for any evidence it affords regarding chromosome structure, but because of its bearing on the theory of spindle origin set forth by DEVISÉ (5) for microsporocytes and by ROBYNS (34, 35) for somatic cells. In the root tip nuclei of *Hyacinthus* and *Vicia*, ROBYNS found that as the prophase comes to a close the nuclear membrane contracts about the chromosomes, leaving behind two polar caps of karyolymph which become the spindle cones. Hence the spindle is composed primarily of karyolymph, although this may be modified in some way through interaction with non-nuclear constituents.

In *Podophyllum* essentially the same series of changes has been observed. The nucleus shown in fig. 49, however, is exceptional in that its membrane has disappeared with little or no shrinkage. The contour of the nucleus remains as it was in the preceding late prophase stage, except for a slight extension at the lower pole. The karyolymph shows the striations characteristic of fixed spindle substance. The insertion points of the chromosomes have assumed their metaphasic positions in the equatorial plane, and show the small projections which suggest the beginning of anaphasic movement. The condition illustrated in this figure seems to leave no escape from the view that the spindle in such cells is composed primarily of karyolymph.

With regard to the structure of the chromosomes in the metaphase, the most instructive figures so far observed are those in *Trillium grandiflorum*, the plant in which GRÉGOIRE and WYGAERTS (10) described "alveolar" chromosomes at this stage. In fig. 50 there appears to be but one thick chromonema in each half-chromosome, just as one is often led to believe for other stages (figs. 4, 14, 29, etc.). In fig. 51, however, each half shows two well separated chromonemata; this stage may be considered to follow that of fig. 47. Still more striking are the conditions illustrated in figs. 52–54: here the chromonemata are somewhat thicker and more nearly parallel, which gives each half-chromosome the aspect ordinarily presented by a whole chromosome in late prophase (cf. fig. 45). A further indication of duality in each half-chromosome is frequently found at the insertion point. In fig. 55 a terminally inserted chromosome of *Trillium* shows four peglike projections, two on each half. Similarly, subterminal insertion points may show four small bridges across a relatively achromatic region (fig. 56). Only a few observations of this nature have been made; hence no decided statement regarding the significance of such appearances is warranted. A further special study of the chromonemata at constrictions and insertion points would probably yield information of much value.

The structure illustrated in figs. 50–54 may be seen not only in material specially treated, but occasionally also in material prepared in the usual manner. These five figures are all from material of the latter kind, the fixation being made in Benda's fluid; and with the exception of figs. 52 and 53, all are from different roots. A full explanation of the variation in these metaphase chromosomes is not yet at hand, but I have made use of the provisional hypothesis that the two chromonemata show different degrees of approximation or separation under different conditions of growth and fixation, and that the processes concerned in their doubling vary in some measure with respect to the phases of the mitotic cycle. A certain amount of variation of this kind occurs in nuclei of one preparation, as is indicated for stages other than the metaphase; and there is even more diversity when one compares different lots of material of the same species, and especially of different species. The most advanced stage of doubling in metaphase half-chromosomes observed is in *Trillium*.

In view of what has frequently been claimed regarding the dependence of the division of other organic units upon their growth and absolute mass, I have been led to inquire whether the precocity in the division of the chromonemata in the chromosomes here described may not be related to their unusually large size. Suppose, for example, that the morphological division of the chromonema occurs when its growing mass reaches a certain critical value, or when the multiplying molecular or colloidal units of which it is composed reach a certain number, this value or number not necessarily being precisely the same for all cases. In a large chromosome we might expect this critical point to be reached at an earlier stage of the mitotic cycle than in a small chromosome, inducing the doubling of the chromonema further in advance of the time at which its halves are to separate. If this were so, doubling might be detected at various mitotic phases in different tissues or in different species; or, it is possible that some condition peculiar to the prophase may determine the rearrangement of materials which results in a visible doubling of the chromonema only in that phase, even if the mass of substance or the number of constituent units has reached the critical value at an earlier stage. In the latter case the visible division of the chromonema would always be prophasic, although in large chromosomes it might appear one full mitotic cycle earlier than in small chromosomes. Accordingly, small chromosomes may be chromatically double in the metaphase, but not quadruple as in the large chromosomes of *Trillium*. Whatever conception of chromosome structure one may derive from the study of large chromosomes, however, there is always open the possibility that small chromosomes may have that same structure but do not reveal it to the eye because their constituent elements are smaller or more compactly arranged. This touches such significant questions as that of the relative number and arrangement of genes in chromosomes of different size, which makes it all the more desirable to obtain some evaluation of the foregoing speculations.

Conclusions

1. The large somatic chromosomes of the five plants studied consist of two principal morphological constituents. These differ decidedly in their affinity for stains during late telophase, interphase, metabolic stage, and early prophase, but not so greatly during late prophase, metaphase, anaphase, and early telophase.

2. The more chromatic constituent of each chromosome persists throughout the mitotic cycle in the form of two chromonemata. The less chromatic constituent forms a matrix in which the chromonemata lie during most of the cycle. It is uncertain whether it remains distinct from the matrix substance of the other chromosomes and from the karyolymph during the interphase, or loses its identity between mitoses.

3. The series of alterations undergone by the two principal constituents of the chromosome through the successive phases of the mitotic cycle are summarized with the aid of a diagram in the preliminary statement near the beginning of the paper. From that

statement it follows that the respective matrices of two sister chromosomes separating in the anaphase are defined by a division at the end of the immediately preceding prophase; whereas, their respective chromonematic constituents are defined by a division in the second preceding prophase.

4. It is suggested that if the division of nuclear elements is related in a definite way to their growth and mass, the stage in the mitotic cycle at which the chromonema becomes longitudinally doubled may not be precisely the same in all nuclei. Hence the interpretation given here for large chromosomes, in which the chromonema divides one complete mitotic cycle in advance of the matrix, should not be extended to small chromosomes without a special study of appropriate material.

5. Intensive studies with more refined methods should be made of the following uncertain points: (1) the precise stage at which the division of the chromonema begins in chromosomes of all sizes; (2) the manner in which this division may involve any smaller elements; (3) the morphology of the chromonema with respect to chromomeres; (4) the arrangement and behavior of chromonemata with respect to constrictions, satellites, and insertion points; (5) the influence of cultural conditions and fixation upon the spatial arrangement of the chromonemata in a chromosome; (6) possible alterations in the length of the chromonema during the mitotic cycle; (7) the measure in which the matrix of each chromosome retains its identity through the interphase; (8) the origin of the extra-chromosomal karyolymph so abundant in the late prophase, and its relation to the matrix substance of the chromosomes; (9) the effect of a larger variety of conditions and reagents upon the relative chromaticity of the chromosomal constituents; (10) the precise rôle of the nucleolus; (11) the physical and chemical characteristics of the chromosomal constituents at different stages; (12) the relation of the chromonemata to the matrix through meiosis, with special reference to the mechanism of crossing-over.

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EXPLANATION OF PLATES XXII–XXIV

All figures were drawn with the aid of an Abbe camera lucida, the mirror distance being increased in most cases to give greater enlargement. The drawings have been reduced one-half in reproduction, and show magnifications as follows: figs. 1, 8, 20, 26, 39 and 49, ×1750; figs. 17, 18, ×1900; all other figures, ×3500. The following lenses were used for critical examination: Zeiss apochromatic objective, 2 mm. N.A. 1.40; compensating oculars 6, 8, 12, and 18; immersed achromatic condenser, N.A. 1.40. Special care was taken to obtain critical illumination. Figs. 17 and 18 are reprinted from the paper of 1920 (42).

PLATE XXII

Fig. 1.—*Vicia:* general appearance of long and short chromosomes in anaphase.

Fig. 2.—*Vicia:* anaphase chromosomes, showing indications of doubleness at distal end (Benda).

Fig. 3.—*Trillium:* portions of anaphase chromosomes, showing doubleness (Benda).

Fig. 4.—*Vicia:* anaphase chromosomes with chromonemata (Benda).

Fig. 5.—*Allium:* anaphase chromosomes with chromonemata (method 20).

Fig. 6.—*Allium:* slightly later than fig. 5; note duality in *b* (method 20).

Fig. 7.—*Allium:* tassement polaire; two chromonemata in upper chromosome, apparently only one in lower (method 20).

Fig. 8.—*Vicia:* general aspect of medium telophase (Benda).

Fig. 9.—*Vicia:* middle chromosome of fig. 8.

Figs. 10, 11.—*Vicia:* telophase chromosomes, showing two chromonemata in some regions (Benda).

Fig. 12.—*Tradescantia:* telophase chromosome with two chromonemata (Flemming).

Fig. 13.—*Tradescantia:* same, with apparently one chromonema (Flemming).

FIG. 14.—*Allium:* telophase, with apparently one chromonema (method 4).

FIG. 15.—*Vicia:* interphase, with anastomosed chromonemata apparently single (Benda).

FIG. 16.—*Allium:* portion of interphase nucleus prepared by method 4.

FIG. 17.—*Tradescantia:* general aspect of interphase nucleus (Flemming).

FIG. 18.—*Tradescantia:* general aspect of metabolic stage (Flemming).

PLATE XXIII

FIGS. 19–38: early prophase.

FIG. 19.—*Vicia:* portions of two isolating chromosomes (Benda).

FIG. 20.—*Podophyllum:* general aspect of early prophase nucleus (Benda).

FIG. 21.—*Vicia:* duality visible at some points (Benda).

FIG. 22.—*Vicia:* chromonemata well separated in places (Benda).

FIG. 23.—*Vicia:* matrix stained; interpretation doubtful (Benda).

FIG. 24.—*Podophyllum:* chromonema irregular and apparently single in many regions (Benda).

FIG. 25.—*Podophyllum:* end of chromosome in which chromonema is clearly double in most destained portion, apparently single in middle portion, and invisible in darkest portion (Benda).

FIG. 26.—*Podophyllum:* general aspect of nucleus in "spiral stage" (Benda).

FIG. 27.—*Vicia:* spiral chromonema apparently single (Flemming).

FIG. 28.—*Vicia:* same (Benda).

FIG. 29.—*Trillium:* spiral chromonemata appearing double only in certain regions (Benda).

FIG. 30.—*Trillium:* same stage as fig. 29, but with doubleness showing clearly (Benda).

FIG. 31.—*Podophyllum:* interpretation of uppermost chromosome doubtful; middle chromosome curiously extended; lowermost chromosome showing doubleness clearly (Benda).

FIGS. 32, 33.—*Tradescantia:* doubleness apparent at some points (Flemming).

FIGS. 34, 35.—*Tradescantia:* doubleness evident almost throughout (Flemming).

FIGS. 36, 37.—*Vicia:* specially clear examples of doubleness in early prophasic chromonemata (Benda).

FIG. 38.—*Vicia:* double chromonemata tending to straighten while still very slender (Benda).

PLATE XXIV

FIG. 39.—*Podophyllum:* general aspect of nucleus in middle prophase (Benda).

FIGS. 40–42.—*Vicia:* chromosomes in middle prophase; note wide divergence of straightened chromonemata in fig. 40 and chromomeres in fig. 42 (Benda).

FIG. 43.—*Allium:* chromonemata becoming contorted as chromosomes shorten in later prophase (method 20).

FIG. 44.—*Vicia:* late prophase; chromonemata contorted and showing suggestions of possible subdivision at points indicated by arrows (Benda).

FIG. 45.—*Podophyllum:* late prophase chromosome (Benda).

FIG. 46.—*Tradescantia:* fragment of late prophase chromosome showing two chromonemata (Flemming).

FIG. 47.—*Trillium:* portions of late prophase chromosomes, showing undivided matrix with two chromonemata, in each of which duality is suggested at certain points (Benda).

FIG. 48.—*Trillium:* very late prophase; matrix divided (Benda).

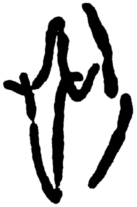
FIG. 49.—*Podophyllum:* transition from late prophase to metaphase, an unusual aspect; karyolymph forming spindle while prophasic contour of nucleus is retained; insertion points of chromosomes arranging in equatorial plane (Benda).

FIG. 50.—*Trillium:* two double metaphase chromosomes, with chromatic substance forming apparently single but heavy chromonema in each half (Benda).

FIG. 51.—*Trillium:* portion of metaphase chromosome, with two well separated chromonemata in each half (Benda).

FIGS. 52–54.—*Trillium:* metaphase chromosomes with well developed chromatic duality in each half (Benda).

FIGS. 55, 56.—*Trillium:* terminal and subterminal insertion points of metaphase chromosomes, showing indications of duality in each half (Benda).



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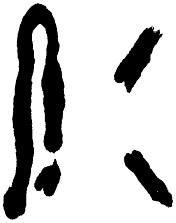

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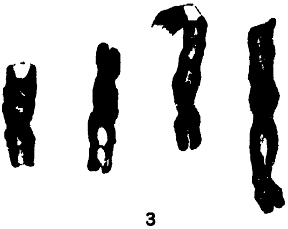

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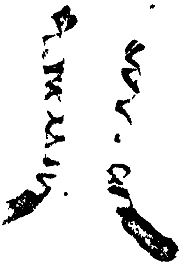

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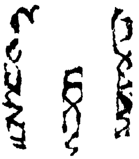

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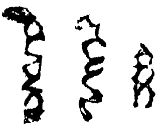

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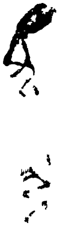

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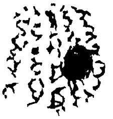

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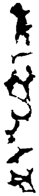

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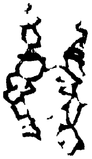

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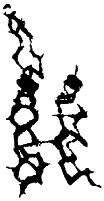

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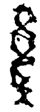

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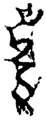

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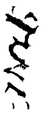

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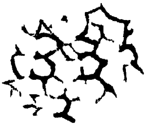

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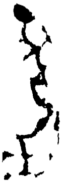

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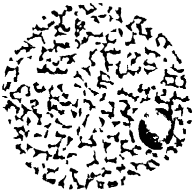

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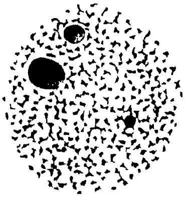

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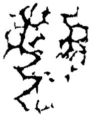
SHARP on CHROMOSOMES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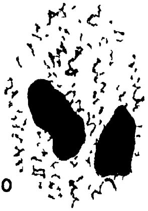
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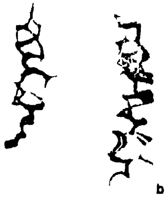

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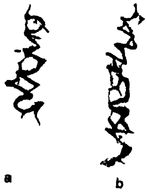

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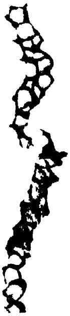
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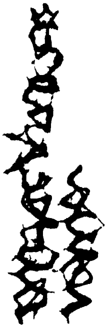
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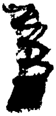
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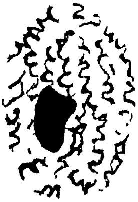
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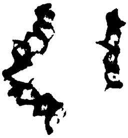
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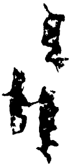
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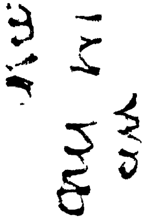
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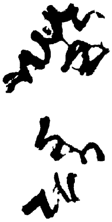
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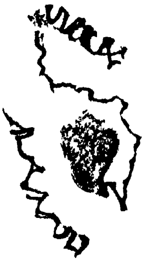
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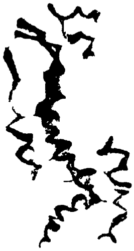
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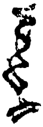
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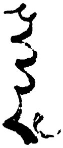
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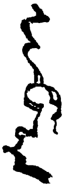
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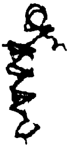
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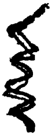
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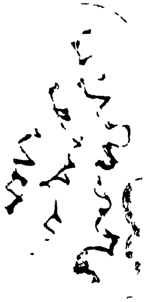
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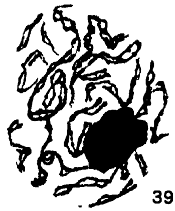
SHARP on CHROMOSOMES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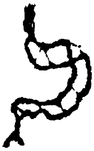
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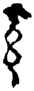
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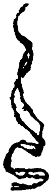
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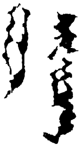
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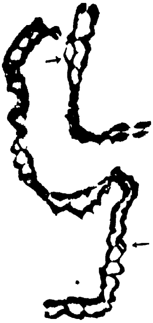
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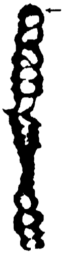
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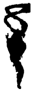
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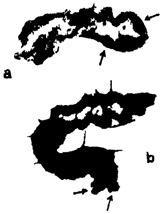

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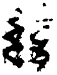
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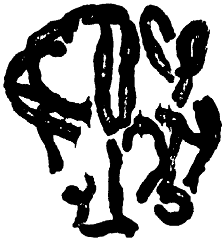
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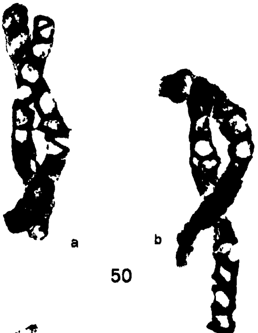

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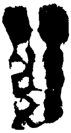
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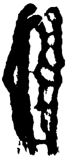
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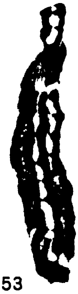
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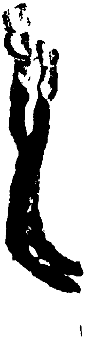
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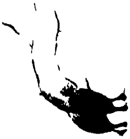
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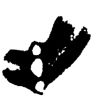
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SHARP on CHROMOSOMES

CYTOLOGICAL STUDIES IN THE BETULACEAE

II. CORYLUS AND ALNUS[1]

ROBERT H. WOODWORTH

(WITH FIFTY FIGURES)

The materials and methods used in this study are described in a previous paper (WOODWORTH 19).

CORYLUS (TOURN.) L.

There is at hand no evidence which would indicate polyploidy in this genus. Ten species, four varieties, and three known hybrids have been examined, and all show the haploid number of chromosomes to be fourteen. This condition is interesting in view of the fact that the group is so closely related to the highly polyploid genus *Betula*.

In species of *Betula* the size of the mother cell is in general proportional to the size of the chromosome complement. In *Corylus* the mother cells are as large or larger than those of *B. lutea*, which has forty-two chromosomes and the largest P.M.C. of any species of *Betula* examined. Also the chromosomes of *Corylus* are by far the smallest seen in the Betulaceae, which means that the karyoplasmic ratio (WILSON 15) in *Corylus* is several times smaller than in *Betula*. It may be worth while to note, therefore, that while this ratio is definite throughout the polyploid species of *Betula*, it is not at all constant in a comparison of the two genera.

Several times the cytoplasm has been noted to consist of large dense spheres, up to one-fifth the diameter of the cell in size.

Apparently natural hybrids are easily formed among the species. REHDER (12) has described *C. avellana*×*colurna*, *C. tibetica*× *avellana*, and *C. chinensis*×*avellana*. There is a *C. americana* × *pontica* in the Arnold Arboretum. GOESCHKE (7), in his economic treatment of the hazel nuts, presents a "Pomologische Beschreibung der Haselnüsse" in which he describes and illustrates eighty-seven sorts of *Corylus* plants which bear different types of nuts. WINKLER

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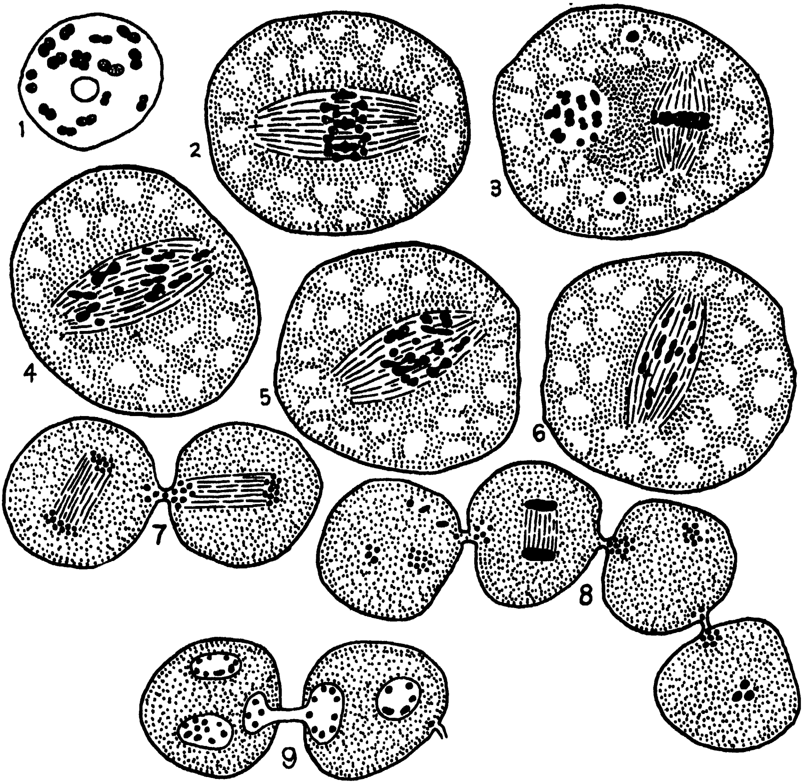
[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

(18), monographing the Betulaceae, recognized in *Corylus* eight species, fifteen varieties, and two hybrids. Many of the horticultural varieties, listed by GOESCHKE, may have arisen through hybridization.



FIGS. 1–9.—Pollen mother cells and chromosome groups of species of *Corylus* during meiosis; ×2300 (except figs. 7–9): fig. 1, *C. heterophylla*, nucleus at diakinesis showing 14 bivalent chromosomes; fig. 2, *C. americana*, heterotypic metaphase; fig. 3, same, homeotypic metaphase showing extruded chromatin; fig. 4, *C. maxima* var. *atropurpurea*, heterotypic division; bivalent and univalent chromosomes lagging on spindle; fig. 5, *C. cornuta*, heterotypic division showing bivalents and univalents; fig. 6, *C. americana*×*pontica*, heterotypic division showing bivalents and univalents lagging on spindle; fig. 7, same, cytomyxis and chromosome migration at heterotypic anaphase (×1200); fig. 8, *C.* no. 9 of Vollertsen, cytomyxis and chromosome migration between four mother cells (×1200); fig. 9, *C. cornuta*, cytomyxis and nuclear migration (×1200).

The genus is characterized by more or less normal meioses, which is to be expected in view of the constant number of chromosomes. If the group was typically heterozygous, a considerable degree of normality of the reduction division might prevail. Diakineses appear to consist of normal pairing, although it has been somewhat difficult to find examples of this stage. Sometimes a fusion of gemini has taken place. The heterotypic metaphase, in practically all the species examined, exhibits a pairing of certain of the bivalent chromosomes. Two or three pairs of the gemini unite, appearing in all stages, from a mere touching to complete resolution into one large chromosome mass.

Cytomyxis and chromatolysis also appear in most of the preparations. An interesting phase of the action of these plasma bridges is the migration of part or the whole of the chromosome complement from one mother cell into the cytoplasm of a neighboring mother cell.

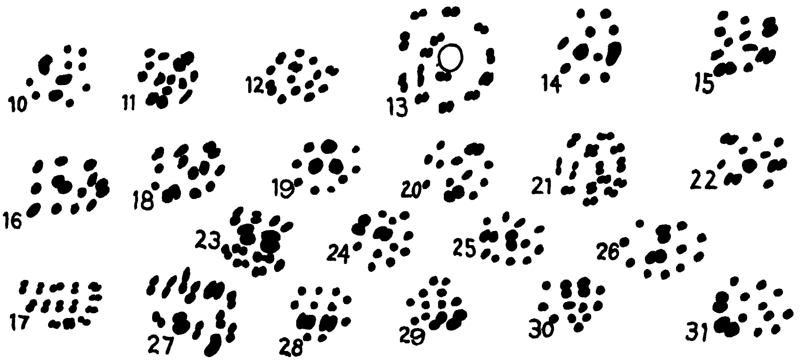
Corylus americana Walt., X-14 (meiosis somewhat abnormal).—Both diakineses and heterotypic metaphases occur with apparently eleven chromosomes, but there are always three large clumps, each of which is composed of two gemini fused (fig. 10). The chromosomes are regular in their movements during the first division (fig. 2). The homeotypic metaphase frequently shows chromosomes extruded into the cytoplasm (fig. 3). The chromosome count of the spindle in plate view clearly indicates that these bodies are chromosomal in nature, since it does not have its full complement.

Cytomyxis is frequently seen at diakinesis, heterotypic anaphase, and at interkinesis; and is often accompanied by the migration of chromosomes across the plasma bridges (fig. 7). This is one of the possible origins of an increase in chromosome number.

C. americana×*pontica*, X-14 in both parents and in this hybrid (meiosis somewhat abnormal).—During the early heterotypic spindle the chromosomes appear as bivalent and univalent, and are distributed all over the fibers before gathering at the metaphase plate (fig. 6). The heterotypic metaphase plates exhibit all stages of the coalescence of gemini. Some plates show fourteen chromosomes very distinctly, while others show all phases from the approximation of two pairs of bivalents to their complete fusion (fig. 11). The second division is normal.

Cytomyxis is present, and the pollen is mostly normal, about 2 per cent being defective. A few microcytes appear and are no doubt caused by unequal distribution of chromosomes or by chromosome loss by cytomyxis.

C. avellana L., X-14 (meiosis mostly normal).—This species produces the filbert of commerce.



FIGS. 10–31.—Fig. 10, *C. americana*, heterotypic metaphase plate, showing characteristic condition: 3 of gemini fused, forming quadrivalents, making chromosome count appear as 11; fig. 11, *C. americana*×*pontica*, heterotypic metaphase plate; fig. 12, *C. pontica*, heterotypic metaphase plate, some chromosomes already split for second division; fig. 13, *C. avellana*, diakinesis; fig. 14, same, heterotypic metaphase plate; fig. 15, *C. avellana* var. *pendula*, heterotypic metaphase plate; fig. 16, *C. colurna*, heterotypic metaphase plate; fig. 17, *C. heterophylla*, interkinesis; fig. 18, *C. heterophylla* var. *sutchuensis*, heterotypic metaphase plate; fig. 19, *C. maxima*, heterotypic metaphase plate; fig. 20, *C. maxima* var. *atropurpurea*, heterotypic metaphase plate; fig. 21, *C. cornuta*, diakinesis; fig 22, same, heterotypic metaphase plate; fig. 23, same, diakinesis; fig. 24, same, heterotypic metaphase plate; fig. 25, *C. sieboldiana*, heterotypic metaphase plate; fig. 26, *C. sieboldiana* var. *mandshurica*, heterotypic metaphase plate; fig. 27, *C. spinescens*, diakinesis; fig. 28, same, heterotypic metaphase plate; fig. 29, *C. tibetica*, heterotypic metaphase plate; fig. 30, *C. villmorinii*, heterotypic metaphase plate; fig. 31, *C.* no. 9 of Vollertsen, heterotypic metaphase plate.

Diakinesis clearly shows fourteen gemini (fig. 13). Heterotypic metaphase plates show all conditions, from fourteen separate gemini to thirteen, twelve, and eleven gemini with one, two, and three large quadrivalent groups. One cell had nine bivalents, one large quadrivalent, and one very large hexivalent (fig. 14).

Cytomyxis noted at heterotypic metaphase. Sometimes as many as twelve small dark bodies are seen about the periphery of the cell during the heterotypic division and interkinesis. These are not chromosomes, the full complement being on the spindle.

C. tibetica×*avellana* (see *C. spinescens* Rehd.).

C. chinensis×*avellana* (see *C. vilmorinii* Rehd.).

C. avellana var. *pendula* Goeschke,[2] X-14 (meiosis normal).—Three gemini are commonly fused at the heterotypic division (fig. 15). Cytomyxis was noted.

C. colurna L., X-14 (meiosis regular).—Two bivalent chromosomes commonly fused during heterotypic division (fig. 16).

Cytomyxis noted at interkinesis. Two P.M.C.'s were seen in close contact, being contained in but one callose sheath.

C. cornuta Marsh. (*C. rostrata* Ait.).—This species is commonly known as *C. rostrata* Ait., but the other name must be accepted because Marshall (9) published it in 1785, while Aiton's (7) publication was dated 1788.

X-14 (meiosis abnormal).—During meiosis some of the chromosomes are tardy in their actions on the spindle (fig. 5). Figs. 21 and 23 show the diakinesis, the former with fourteen gemini showing no fusion (one bivalent has already split for the second division), and the latter with two pairs of gemini fused, making quadrivalents.

The heterotypic metaphase plates give counts of fourteen, thirteen, and twelve, the two latter being due obviously to fusion of bivalents (figs. 22, 24). In fig. 22 one pair has resolved into one large chromosome, while three other pairs are close together. In fig. 24 two pairs have fused but their identity is still apparent.

The homeotypic metaphase plates have given counts of eight, nine, ten, twelve, and fourteen. These counts, other than fourteen, may be due to further coalescence of chromosomes or to chromosome loss by cytomyctic migration. Occasionally a chromosome was seen to be extruded into the plasma. This variation in chromosome number is clearly reflected in the size of the pollen grains, which occur as microcytes and intermediate sizes up to the full-sized grains. Five per cent of the pollen is defective.

Cytomyxis was noted frequently. Fig. 9 shows a typical example where there seems to be a migration of chromosomes from one cell into the next.

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[2] This tree was labeled "natural variety."

C. heterophylla Fisch., X-14 (meiosis normal).—Fig. 1 shows diakinesis with fourteen gemini, one of which has split already for the second division. Fig. 17 illustrates the chromosome complement of one of the interkinetic nuclei.

Cytomyxis and chromatolysis occur during early meiosis.

C. *heterophylla* var. *sutchuensis* Franch., X-14 (meiosis normal).—Two pairs of gemini are consistently fused during the heterotypic division (fig. 18). All phases are seen, from mere approximation to complete resolution into one large mass. The cytoplasm shows large dense areas which are sometimes as broad as the spindle.

C. maxima Mill.,[3] X-14 (meiosis normal).—Three pairs of the gemini commonly fuse completely, so that the metaphase plate appears to have eight small chromosomes and three large ones (fig. 19). Occasionally a bivalent is tardy in its actions on the first spindle. Cytomyxis was noted. Pollen was entirely perfect.

C. maxima var. *atropurpurea* Dochnahl., X-14.—This variety is of garden origin. It accordingly shows some of the abnormal cytological characteristics which usually mark such plants.

In the early heterotypic division there are univalent chromosomes on the spindle but they do not cause later irregularities of meiosis. All the chromosomes are tardy in the early heterotypic spindle (fig. 4). There are usually two bivalents fused during the first division (fig. 20).

The pollen is characteristically good. Occasionally a few small or defective grains were noted.

C. pontica Koch., X-14 (meiosis entirely normal).—Fig. 12 shows fourteen distinct chromosomes, four of which have already split for the homeotypic division.

C. sieboldiana Blume., X-14 (meiosis normal).—Two pairs of gemini are usually fused (fig. 25). In homeotypic metaphase two mother cells were completely fused together, forming a large cell with four spindles.

C. sieboldiana var. *mandshurica* (Bl.) Schneid., X-14 (meiosis mostly normal).—In early heterotypic division chromosomes are occasionally tardy in their behavior on the spindle. Two pairs of gemini are consistently fused (fig. 26).

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[3] Hybrids between this and *C. avellana* are commonly planted for the nuts.

C. tibetica Batalin., X-14 (meiosis normal).—Two gemini fused during heterotypic division (fig. 29).

× *C. spinescens* Rehd. (*C. tibetica*×*avellana*), X-14 (meiosis normal).—Both diakinesis and heterotypic metaphase plates show two gemini to be consistently fused (figs. 27, 28). Only 30 per cent of the pollen grains are full-sized. A large proportion are very small and many are intermediate in size. Five per cent of the pollen is morphologically sterile.

× *C. vilmorinii* Rehd. (*C. chinensis*×*avellana*), X-14 (meiosis normal).—Two gemini fused in heterotypic division (fig. 30). Cytomyxis was noted. Ten per cent of the pollen consists of microcytes, most of which are shriveled and without contents.

Corylus no. 9 of VOLLERTSEN's improved filbert varieties (labeled *C. vollertseni* in the Arnold Arboretum), X-14 (meiosis somewhat abnormal).—Heterotypic metaphase plates show two or three bivalents fused (fig. 31). In the early heterotypic division the chromosomes are tardy in their behavior, but in the anaphase they appear to migrate sharply to the poles. The homeotypic divisions are normal except that they frequently show one or two extruded chromosomes in the plasma.

Cytomyxis and chromatolysis are marked in this species, especially at prophase and interkinesis. In many anthers the P.M.C.'s are connected by cytoplasmic bridges, and during the early heterotypic metaphase there is a wholesale interchange of chromosomes. Fig. 8 shows a typical case of such actions at the heterotypic anaphase. The pollen is largely perfect. A few smaller grains appear.

ALNUS (TOURN.) HILL

This genus is of particular interest from the cytological viewpoint on account of the marked diversity and intermingling of specific morphological characters in *Alnus incana* and *A. rugosa*. Polyploidy is represented by two tetraploid species, *A. glutinosa* of Europe and *A. japonica* from Japan.

Cytomyxis was noted in all species studied. Kinoplasm is apparent in all of the figures.

A. crispa Pursh var. *mollis* Fernald, X-14 (meiosis normal).—Fig. 47 shows a late diakinesis with fourteen bivalent chromosomes.

A. maritima (Marsh.) Muhl., X-14 (meiosis normal).—Fig. 46 shows the heterotypic metaphase plate with fourteen chromosomes. Frequently one of the gemini is very late in separating. In no instance, however, has this been noted to persist long enough to cause non-disjunction. This species is commonly attributed to NUTTALL, but he (10) distinctly notes that MUHLENBERG is the original author.

A. incana (L.) Moench., X-14 (meiosis normal).—Fig. 43 shows the normal metaphase spindle of the first division; fig. 44 shows the anaphase of the same division. Fourteen chromosomes are clearly seen at each pole. Fig. 45 shows the two homeotypic spindles. No cells showing abnormalities of meiosis have been seen in material of this species. Small darkly stained bodies have frequently been noted about the periphery of the cell. They are minute in comparison with a chromosome, and are clearly not chromosomal in nature.

There is some question as to just where the specific lines of this species and *A. rugosa* begin and end. The material here reported has all been taken from specimens which exhibit the characters set forth in the seventh edition of GRAY'S Manual. When these characters are all present, the bark on the older parts of the shrub shows conspicuous, raised, horizontal lenticels.

A. rugosa (DuRoi) Spreng. In bulletin no. 145 of the Vermont Agricultural Experiment Station it is stated that *A. incana* and *A. rugosa* intergrade around the lake shores. In GRAY'S Manual the following statement appears under *A. rugosa:* "Many shrubs near the northern limits of this range appear intermediate between this and the last species *(A. incana).*" WINKLER (18) lists hybrids between *A. glutinosa* and *incana*, *A. glutinosa* and *rugosa*, and *A. incana* and *rugosa.* The writer has examined many stands of alder in Maine, and very frequently it was impossible to place an individual as typical *A. incana* or *A. rugosa.* Professor FERNALD has kindly shown the writer collections of *Alnus* from various parts of northeastern America which are of the *incana-rugosa* group, but which possess characters that do not allow them to be classed with either one species or the other. With our present understanding of this complex, it is possible to say of a plant one of three things: it is *A. incana*, it is *A. rugosa*, or it is of them but not one of them.

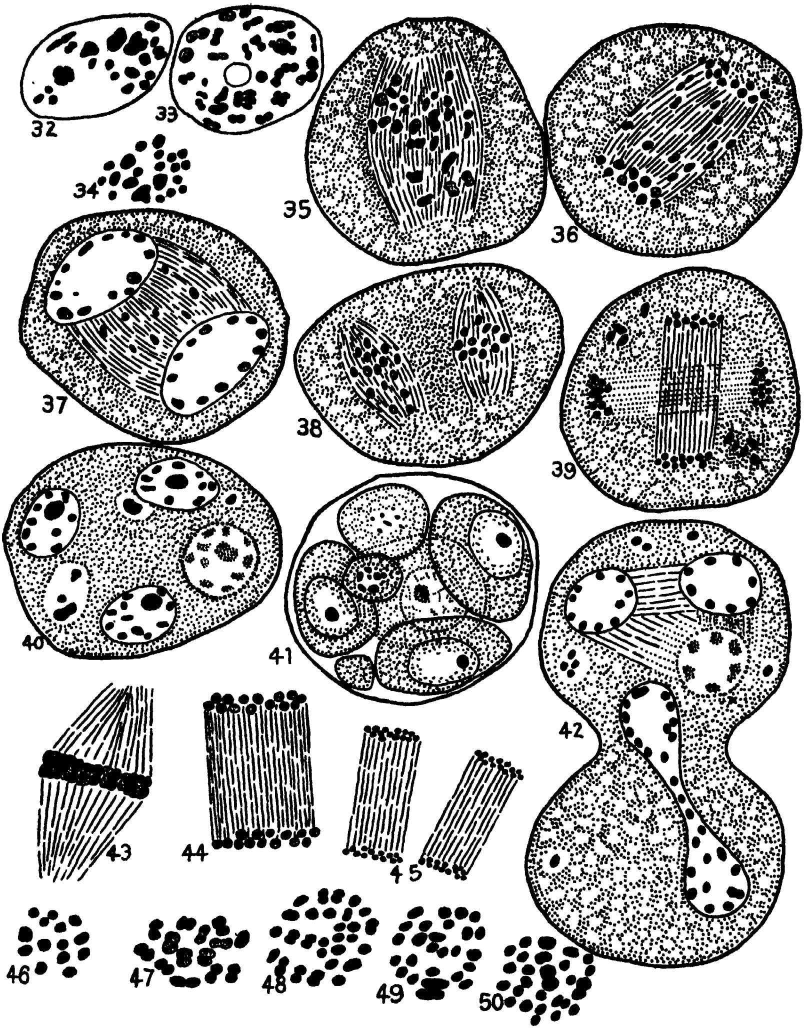
In writing of *A. rugosa*, the writer refers to the plant which grows in northern and middle New England. The opportunity to study material of *A. rugosa* from the southern United States has not yet been realized; there the species may be quite stable.

It is the usual occurrence to find extensive tracts of *A. rugosa* practically destitute of staminate catkins. Clumps of several hundreds of shrubs have been noted to possess but a few of these sterile aments which have 95 per cent of their pollen sterile. Since the plants set very large quantities of seed and there is obviously not nearly enough pollen to fertilize them all, it appears that either they are pollinated by *A. incana* or they form their seed apomictically.

Large numbers of the fertile catkins have been collected and examined cytologically from the time of the shedding of pollen in April up to July 4. In no case had the embryo sac yet been formed, and during May to July the stigmas were black and withered. No indications of pollen grains have been seen on the stigmas during these months, and in no case was the pollen tube observed in the top of the ovary. In view of the large number of preparations examined, it is reasonable to suppose that this plant forms its seeds apomictically. Further investigation has proved this point (WOODWORTH **20**). There is an ever increasing understanding that parthenogenesis is an end product of hybridization. Since there is ample reason to believe that *A. rugosa* is parthenogenetic, there is an accompanying suggestion that the plant is of hybrid origin. The cytological study of microsporogenesis, now to be described, indicates clearly that *A. rugosa* is heterozygous.

The material which is here reported on has been taken from specimens exhibiting the commonly recognized characters of *A. rugosa*. The lenticels also appear to shed some light on the differentiation from *A. incana*. These air passages on the older bark are small, inconspicuous, round, and very close together in vertical rows; in marked contrast to the prominent, horizontal lenticels of *A. incana*, although a few of this latter type are commonly scattered among those typical of *A. rugosa*.

X-probably 14 (meiosis extremely abnormal).—Fig. 33 illustrates the late prophase at the diakinetic stage. This has proved to be a difficult phase to study because of the peculiar pairing and non-



FIGS. 32–50.—Pollen mother cells of species of *Alnus* during meiosis. *A. rugosa:* figs. 32, 33, nucleus at diakinesis; fig. 34, heterotypic metaphase plate; fig. 35, early heterotypic division, no equatorial plate formed, chromosomes distributed all along spindle; fig. 36, heterotypic anaphase, many chromosomes lagging on spindle; fig. 37, heterotypic telophase, several chromosomes excluded from daughter nuclei; fig. 38, early homeotypic metaphase showing unequal distribution of chromosomes; fig. 39, homeotypic anaphase showing extruded masses of chromatin; fig. 40, polycaric (multinucleate) mother cell showing abnormal condition resulting from chromosome extrusion; fig. 41, polyspory; seven pollen grains unequal in size instead of four normal grains; small grains and many large ones degenerate; fig. 42, abnormal behavior of two mother cells. *A. incana:* fig. 43, spindle of heterotypic metaphase (note regularity); fig. 44, spindle of heterotypic anaphase; fig. 45, spindles of homeotypic anaphase; fig. 46, heterotypic metaphase plate of *A. maritima* showing 14 chromosomes; fig. 47, diakinesis of *A. crispa* var. *mollis* showing 14 gemini; fig. 48, heterotypic metaphase plate of *A. japonica* showing 28 chromosomes, some already split for second division; fig. 49, heterotypic metaphase plate of *A. glutinosa* showing 24 bivalents and 2 quadrivalents, fig. 50, heterotypic metaphase plate of same showing 28 chromosomes.

pairing habits of the chromosomes. Some of them have paired, forming gemini, and some of the univalents have already split, as if for the second division, and appear as bivalents. Fig. 32 shows the most easily interpreted diakinesis that has been seen; there are five gemini, one trivalent chromosome body, and fifteen univalents, two of which (on the right) have already split for the mitotic division. This would indicate the diploid number of chromosomes to be twenty-eight.

Fig. 35 shows the usual condition of the heterotypic metaphase. Few gemini are formed and are at the equator, many univalents being scattered all over the spindle. Sometimes, just before the bivalents are separated, the univalents migrate to the equator. Fig. 34 illustrates the metaphase plate of such a spindle. There are seven bivalents and fourteen univalents. Some two dozen metaphase plates of the first division have been counted, a most laborious and trying procedure, most frequently showing twenty-one or twenty-two chromosomes. Six or seven chromosome groups were consistently larger and were undoubtedly bivalent in nature. One count was eighteen and showed a large knot of chromatin which must have been several chromosomes fused together. This fusion of chromosomes is the normal occurrence in *Corylus*. A few counts were twenty-three and twenty-four and showed only three and two of the bivalents. These studies of diakinesis and heterotypic metaphase show the haploid chromosome complement to be probably fourteen.

Fig. 36 shows the anaphase of the first division. The bivalents have separated normally. Some of the univalents have migrated at random, in their whole condition, to the poles; while others have been halved, each half progressing to one of the poles. This causes the chromosome number to be considerably more than twenty-eight, which would of course be the normal number.

Occasionally a few of the univalents do not arrive at the poles but remain on the spindle when the nuclear membranes are formed. This condition is shown by fig. 37. These lagging chromosomes aggregate to form dwarf nuclei and later microcytes.

The regularity of the homeotypic division depends upon the number of univalent chromosomes which have not been divided during the first division. Those which did divide do not undergo division during the second division but lag on the spindle. Fig. 38

illustrates a cell at the beginning of the homeotypic division just before the metaphase. In the first division there was apparently an unequal distribution of the univalents, because one spindle has sixteen chromosomes and the other has twelve. In this case there were no univalents left out of the interkinetic nuclei. The chromosome counts that have been made during the second division have shown from ten to sixteen (most frequently thirteen) chromosomes on each spindle. This inequality is due to the abnormalities of the first division.

Fig. 39 shows the anaphase of the second division with groups of chromosomes in the cytoplasm. These later form microcytes.

Fig. 40, a polycaric mother cell, is seen to be quite abnormal when compared with the usual normal tetrad of a true species. Fig. 42 illustrates an unusual and very irregular broad cytomyctic connection between two mother cells and consequent involvement of the nuclear actions. Such a state has not been noted by the writer in any species which is undoubtedly homozygous.

Fig. 41 shows the end product of these abnormalities. Instead of the normal quartet of pollen grains, polyspory results. The chromosomes left in the cytoplasm have formed microcytes, and the large grains differ in size, due to the loss of part of their legitimate complement. All of the dwarf grains and most of the larger grains degenerate before the pollen is shed, leaving some 5 per cent of the morphologically normal, the rest being represented by shriveled exine coats containing no protoplasm.

These abnormal conditions are now recognized as the peculiarities which obtain in hybrid plants. In a previous paper (WOODWORTH 19) unmistakable evidence in this regard is presented in the studies of two natural hybrids in this same family, *Betula jackii* and *B. sandbergi*. Due to this cytological evidence, to the probability that *A. rugosa* is parthenogenetic, and to the uncertainty of specific lines in the northern parts of its range, it is concluded that *A. rugosa* is of heterozygous origin. It is probably a cross between two species, each of which has the haploid number of chromosomes of fourteen.

A. japonica Sieb. et Zucc., X-28 (meiosis normal).—This species is tetraploid. Fig. 48 shows the heterotypic metaphase plate with twenty-eight chromosomes, some of which are already split for the second division.

A. glutinosa Gaertn., X-28 (meiosis normal).—Figs. 49 and 50 show the heterotypic metaphase plate from polar view. In this species, as occurs typically in *Corylus*, there is a union of two or three pairs of bivalent chromosomes, so that the count appears to be twenty-five or twenty-six rather than twenty-eight. Often this union is quite apparent, as in fig. 50, but sometimes it is not so apparent (fig. 49). Occasionally the union does not occur at all, and twenty-eight chromosomes are distinctly seen.

The early heterotypic spindle appears frequently in the preparations, and was commonly tripolar with the chromosomes scattered all over it. There is an occasional slight tardiness in the action of one or two bivalents during the heterotypic and homeotypic divisions; otherwise the meiosis is quite regular.

Discussion

Light is thrown from several angles on the possible origin of changes in chromosome number. In *Alnus rugosa* the unequal distribution of chromosomes to the poles of the spindles gives rise to gametes without the normal chromosome complement. This is one of the recognized characters of hybrid plants. This same plant produces gametes with less than the full chromosome complement, through a loss of some of the chromosomes into the cytoplasm and subsequent dissolution or incorporation into a small, extra nucleus.

Cytomyxis and migration of chromosomes (WOODWORTH 19) have been seen to have taken place in several of the plants under discussion. When mother cells lose a part or the whole of their chromosome group to neighboring cells, the resulting gametes are abnormal in content.

In *Corylus* all species appear to be diploid, with fourteen chromosomes as the reduced number. Throughout the group there is a pairing of one, two, or three of the bivalent chromosomes, which often makes the chromosome count appear as less than the haploid number. The chromosome groups in figs. 1–31 show the various degrees of fusion of the gemini, from an approximation to an actual resolution into one large mass. *Alnus glutinosa* showed this same coalescence on the part of a few of its gemini; *A. rugosa* exhibits the same thing. This fusion has been noted in no other cells than those which undergo the reduction division. Always there is a definite number of the chromatic elements which participate in this pairing. This indicates that faulty fixation does not explain the phenomenon. Many careful observers have reported this sort of mutual attraction of bivalent chromosomes.

DIGBY (5) found that in *Primula kewensis* (*P. floribunda* × *verticillata*) two of the bivalent chromosomes join together in the heterotype prophase of the P.M.C. nuclei, forming a large quadrivalent chromosome. This union is maintained until its univalent portions separate on the spindle. This does not again take place in the homeotype, nor does it occur in the E.M.C.

In a triploid *Canna*, BELLING (2) found that nine triad chromosomes are formed. They separate into two and one at the anaphase of the first division, and are distributed at random to either pole.

In *Lactuca sativa*, GATES and REES (6) noted in over 50 per cent of the P.M.C.'s a more or less complete coalescence of two or four of the bivalent chromosomes, so that only eight or seven bodies appear on the spindle, and sometimes only five.

BELLING and BLAKESLEE (3) noted in tetraploid *Datura* that during the heterotypic prophase two pairs of gemini unite, forming quadrivalents.

BLACKBURN and HARRISON (4) found two orthoploid series of species in the Salicaceae: 19, 38, 76, and 22, 44. They suggest that the series with 19 as the fundamental number may have arisen from the other series by the fusion of chromosomes.

The *Zea mays* × *Euchlaena perennis* hybrid has thirty chromosomes which at diakinesis unite in trivalents and bivalents, or remain as univalents (LONGLEY 8).

Penstemon isophyllus and *P. campanulatus* show two pairs of chromosomes united in diakinesis, so there are generally observed six smaller and one larger pair of gemini, the latter often evidently consisting of two (WINGE 17).

RANDOLPH and MCCLINTOCK (11), reporting on a triploid form of *Zea mays*, noted at diakinesis trivalents, bivalents, and univalents. DE LITARDIERE (21) derives a nineteen-chromosome species of

Senecio from a twenty-chromosome species by the permanent union of chromosomes.

If one reviews the compilations of the chromosome numbers found in plants (TISCHLER 14, WINGE 16), polyploidy is seen to occur almost universally. Polyploidy is the occurrence of chromosome complements which are multiples of a basic haploid number. In these polyploid series dysploidy is often seen to appear. Dysploidy is the occurrence of chromosome complements which are not multiples of the basic haploid number. Frequently it is apparent that the dysploid species have arisen from the polyploid species, from one of the gametes having one or two chromosomes more or less than the haploid number, due to unequal distribution, or from a fusion of bivalent chromosomes as already described. The more extensive aberrations in chromosome number may have resulted from the participation in fertilization by a microcyte formed from dwarf spindles containing aggregations of extruded chromosomes. SHARP (13) states: "Frequently the functional gametes may show a wide range of variation in their complements, while in other instances only those with certain chromosome assortments will come to maturity and act in syngamy."

In most of the illustrations of P.M.C.'s in this report, it is apparent that the cytoplasm is more dense in the region surrounding the nucleus than it is near the outer portions of the cell. This substance has been considered as distinct from general cytoplasm, and has been variously called kinoplasm, archiplasm, superior protoplasm, and ergastoplasm. In material of the Betulaceae this zone has never appeared to be felted or to have a fibrillar structure. Although several hundreds of sporophytic cells in division have been examined, not one has ever been seen to contain this denser cytoplasm surrounding the nucleus. This would indicate that the perinuclear zone is not an artifact caused by the fixative. WILSON (15) considers "all dualistic hypotheses of the protoplasm . . . . convenient as descriptive devices only." This appears to be a very sane attitude.

Summary

1. *Corylus* exhibits no polyploidy, all species and hybrids having the haploid number of fourteen chromosomes.

2. Throughout the genus there occurs a fusion of one, two, or three pairs of bivalent chromosomes, thus often causing the haploid number to appear to be less than fourteen.

3. Natural hybrids are easily formed. These plants show some of the cytological peculiarities known to be due to heterozygosis.

4. The fundamental number of chromosomes in *Alnus* is also fourteen. Diploid and tetraploid species are described.

5. *Alnus rugosa* shows marked hybrid cytological characters and is considered to be heterozygous. It is also held to form its seed apomictically.

6. *Alnus rugosa* and *A. glutinosa* exhibit fusion of bivalent chromosomes.

7. Cytomyxis and chromosome migration take place in certain species of *Corylus* and *Alnus*.

8. Dysploidy may be due to unequal chromosome distribution, chromosome extrusion, or to cytomyxis and chromosome migration.

9. A perinuclear zone occurs in the P.M.C.'s.

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DEVELOPMENT OF SPOROPHYTE OF MARCHANTIA CHENOPODA

HELEN LOUISE MCNAUGHT

(WITH TWENTY-TWO FIGURES)

Introduction

The morphology of *Marchantia polymorpha* has been the subject of numerous investigations (7, 8, 10, 15, 17, 18, 21). Among these studies, the work of DURAND (10) has been outstanding in his description of the development of the sporophyte. It has been the purpose of the study here recorded to determine whether the sporophyte of *M. chenopoda*, a delicate South American form, varies in its development from that described for *M. polymorpha.*

The material studied was found by Professor GEORGE S. BRYAN, at an elevation of 2000 feet, on the eastern slopes of the Andes Mountains on the trail to the settlement of Pozuzo, in the Province of Huanuco, Peru. Other forms of this same species, which were not included in this investigation, were found at different levels up to 11,000 feet, the size of the thalli varying directly with the increase in elevation. At the lower elevation the thalli were uniformly small, being about 1 cm. in length and 0.4 cm. in width, while those at higher elevations were more than twice that size.

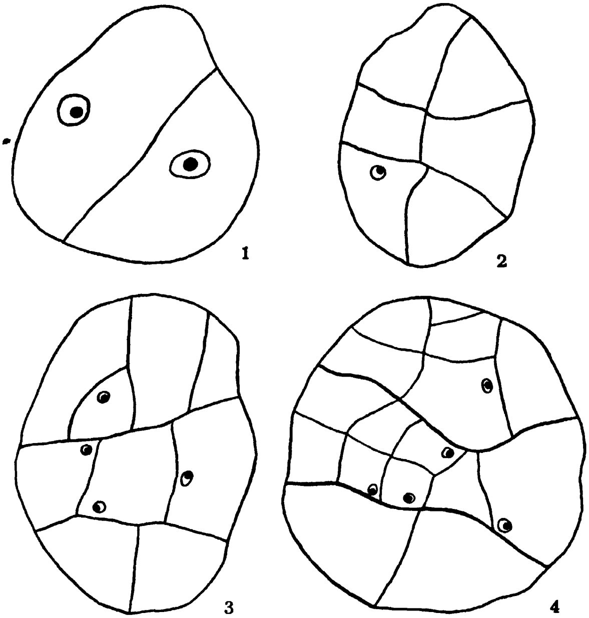
A thorough description of *Marchantia chenopoda* has been given by Professor EVANS (11), who also kindly identified the material used in this study.

The material was collected and killed at various times during the month of June, 1923. The killing fluid was made up in the following proportions: chromic acid 1 gm., glacial acetic acid 1 cc., water 400 cc.

The material was left in this fluid for about eight months, at the end of which time it was washed and run through a series of alcohol-chloroform to pure chloroform. It was then imbedded in paraffin, cut on a rotary microtome from 6 to 10 μ in thickness, and stained with safranin and licht grün.

Development of sporophyte

The first division of the zygote is, as reported for many other Marchantiales, including *Riccia* (3), *Marchantia* (3), and *Preissia* (14), somewhat obliquely transverse to the axis of the archegonium, and results in two approximately equal segments, the hypobasal and the epibasal cells. Fig. 1 shows a first division (unfortunately the only one found) which is decidedly oblique. Judging from the division walls of later embryos, it is highly probable that the first division is usually more nearly transverse. A quadrant division, such as is described for the sporophyte of *M. polymorpha* (10), has not been observed in *M. chenopoda*. Occasionally there are division walls in later embryos which might be interpreted as second division walls



FIGS. 1–4.[1]—Fig. 1, first transverse division of zygote, very oblique; figs. 2–4, further divisions of embryo, showing conspicuous transverse divisions.

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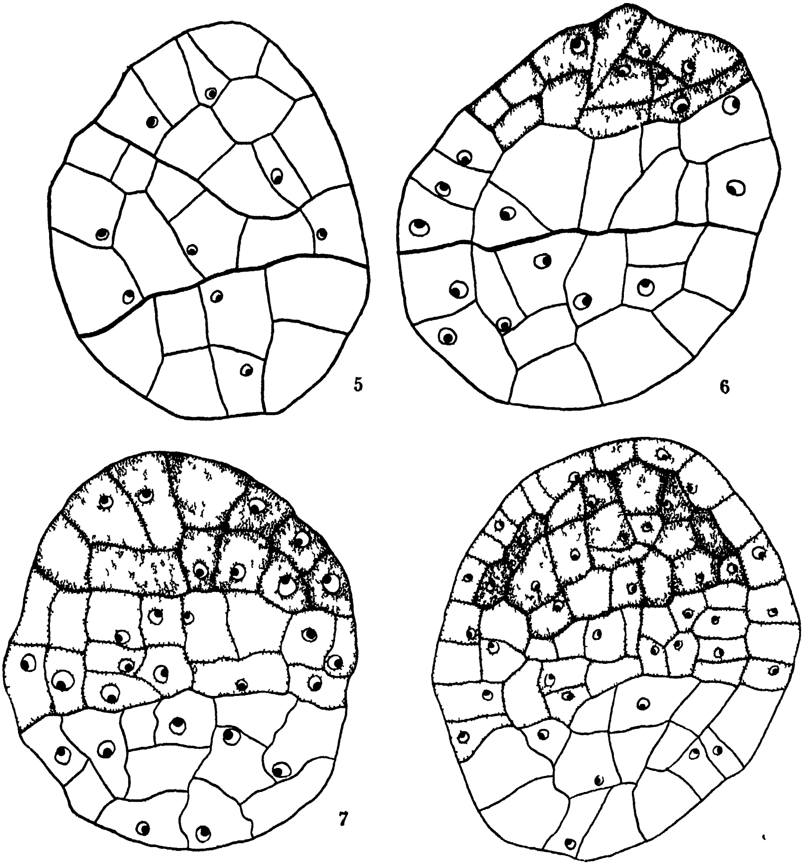
[1] All figures were drawn with the aid of an Abbe camera lucida at table level, and show the following magnifications: figs. 1–8, ×1100; figs. 9–13, 18, ×700; figs. 14, 15, ×550; fig. 16, ×200; fig. 17, ×150; figs. 19–22, ×1500.

at right angles to the first wall, but this is not the most frequent occurrence. There are other divisions which are much more pronounced; namely, two primary transverse divisions which cut the



FIGS 5–8 —Fig 5, further division of embryo; fig 6, early differentiation in staining reaction, showing cells of apical region somewhat darker, fig 7, further differentiation in staining reaction, apical, middle, and basal regions very distinct, fig 8, first appearance of differentiation in apical region between sporogenous region and cells to become capsule wall

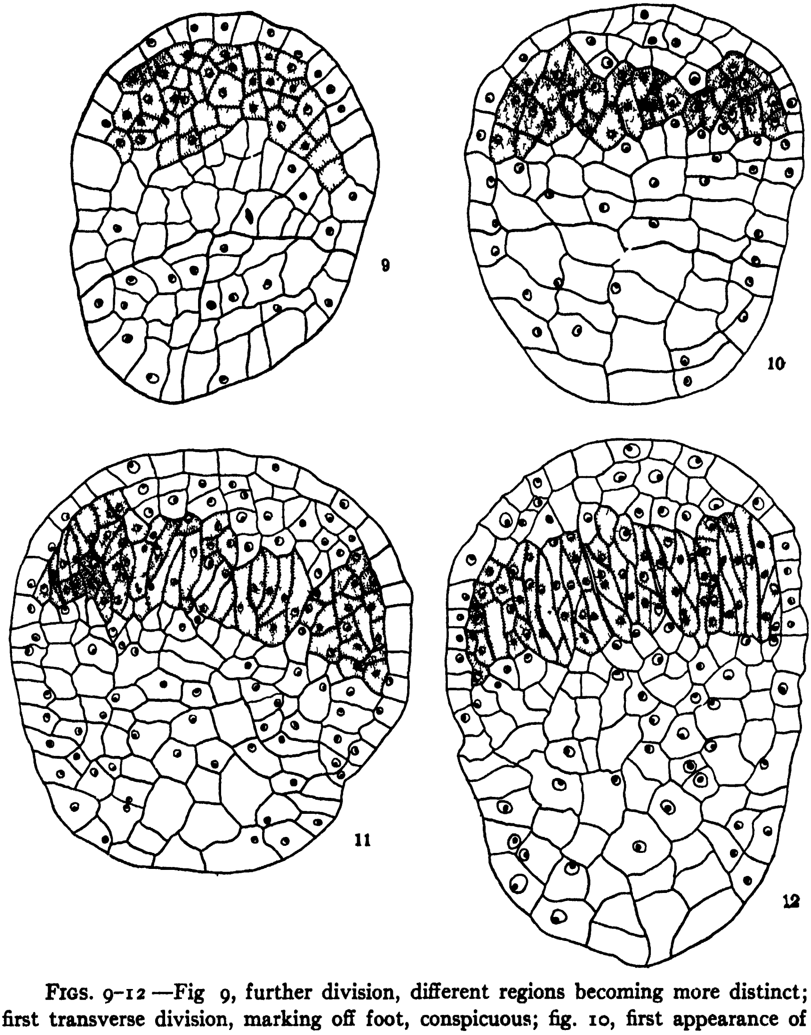
embryo into three distinct parts (figs. 2–5). In the following account these regions will be designated as the apical, middle, and basal regions. As the embryo continues to develop, these three regions remain distinct. From the continuity and the heaviness of the wall which marks off the basal region, it is to be concluded that this is the first transverse division wall. The other, judging from similar evidence, is apparently the result of a transverse division in the epibasal cell. For indication of such divisions note figs. 5, 6, 7, 9, 10, 13. It will be observed that the division which marks off the basal region is the more prominent, probably representing the plane of the first transverse division.

Within the three regions of the embryo anticlinal divisions occur, then periclinal divisions; and then anticlinal, periclinal, and radial divisions in irregular sequence. In a large number of embryos a triangular cell was observed at the apex. That it has a definite function as an apical cell, as is suggested by DUPLER (9) for *Reboulia haemispherica*, is not certain. If so, it seems clear that it does not so function for any considerable length of time.

As divisions continue, and while the embryo is still subspherical in form, a change occurs in the staining reaction of the cells, those in the distal region taking a somewhat deeper stain (fig. 6). With continued division, the cells in the apical region take the heaviest stain, and in the other two regions the staining reaction varies, being a little heavier in the middle region than in the basal (figs. 7. 8). The density becomes greatest in the central cells of the apical region, but the cells in the peripheral layer of this region do not increase in density so rapidly. These outer cells are the ones which will form the capsule wall (fig. 8).

As the density of the inner cells of the apical region increases, they begin to elongate in a direction parallel to the axis of the sporophyte (fig. 11). At the apex there is a small group of cells which remain approximately isodiametric (figs. 10, 11), and take about the same amount of stain as the cells of the capsule wall. This cap of sterile cells persists throughout the life of the sporophyte and undergoes no noticeable change until the walls of these cells take on thickenings similar to those of the capsule wall.

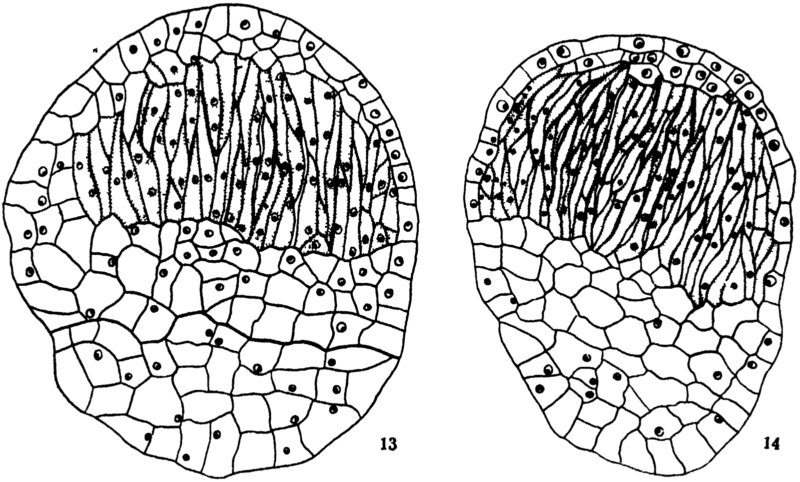
With the continued elongation of the dark-staining (potentially sporogenous) cells, there are cells at the base of this region which do not elongate, and which take a stain heavier than that of the cells in the region of the stalk, but approximately resembling in this respect the cells of the capsule wall. In consideration of the later appearance of capsule wall thickenings in this region of the mature sporophyte, it is probable that the cells in question are those which



FIGS. 9–12.—Fig 9, further division, different regions becoming more distinct; first transverse division, marking off foot, conspicuous; fig. 10, first appearance of sterile, lighter staining cells at apex (to become sterile cap), fig 11, early elongation of cells of sporogenous region; fig 12, further elongation of binucleate cells of sporogenous region; sporophyte longer than broad.

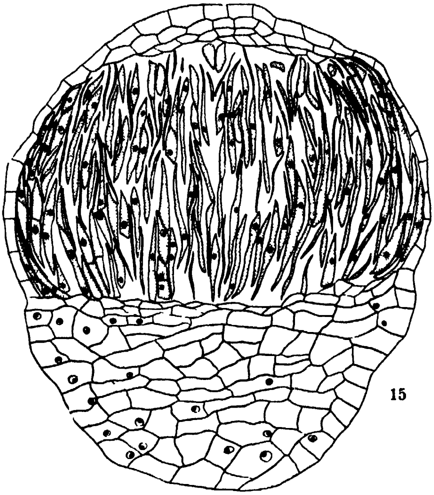
are to form the part of the capsule wall extending across the base of the capsule.

The sporogenous cells elongate greatly (figs. 12, 13). The sporogenous region broadens somewhat and many of the much elongated sporogenous cells are observed to be binucleate, and in some instances appear to contain more than two nuclei (figs. 12, 13). These cells begin to separate from one another laterally (fig. 14). At first no difference between cells that are to become elaters and the true sporogenous cells is to be observed. When the cells are well separated from one another (fig. 15) there appears a difference in width, some being broader than others. All are uninucleate at this time. Those cells which are more broad continue to increase in size, the others remaining very long and narrow. The narrow cells are scattered between the broader ones, being arranged in no definite manner. In the broader cells, which are the true sporogenous cells, transverse cell divisions occur, so that in each a row of from four to eight spore mother cells is formed. These spore mother cells are irregular in shape and are flattened where they come in contact with one another. At this time the capsule is increasing in size, cell division and elongation having occurred in the cells of the capsule



Figs. 13, 14.—Fig. 13, cells of sporogenous region elongated still more, with tendency to separate from one another; conspicuous transverse division marking off foot; fig. 14, further elongation; cells of sporogenous region dividing by oblique transverse wall, to result in uninucleate cells.

wall. The spore mother cells increase slightly in size, and a very heavy wall is formed around each row and between the spore mother cells within the rows (figs. 16, 21). The elaters remain very thin and inconspicuous as regions of slightly granular cytoplasm, with extremely thin walls, lying between the heavy-walled, conspicuous rows of spore mother cells. The spore mother cells now increase in size, and at about the same time the heavy walls which surround and separate them begin to disappear. This disappearance of the walls seems to be a dissolving process, proceeding from the outside inward, until there remain only faint strands of the old wall substance between the spore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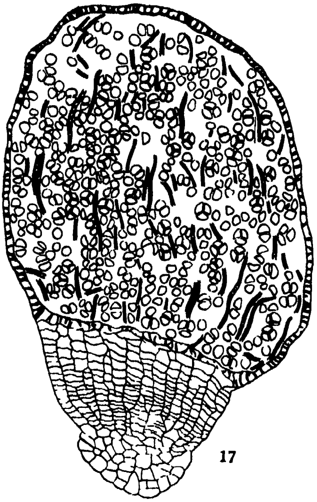
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FIGS. 15, 17.—Fig. 15, cells of sporogenous region well separated; narrow and broader cells observable; note row of small somewhat flattened cells at base of region (to become wall across base of capsule); fig. 17, sporophyte approaching maturity; foot globular; many transverse divisions in seta; thickenings on capsule wall; mature elaters with spiral thickenings; spores separating from tetrads.

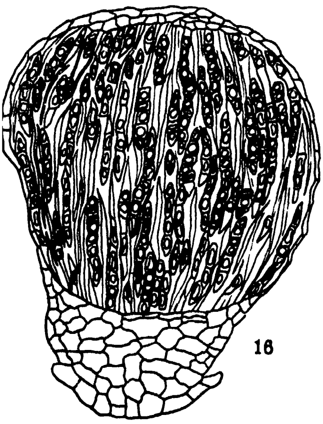
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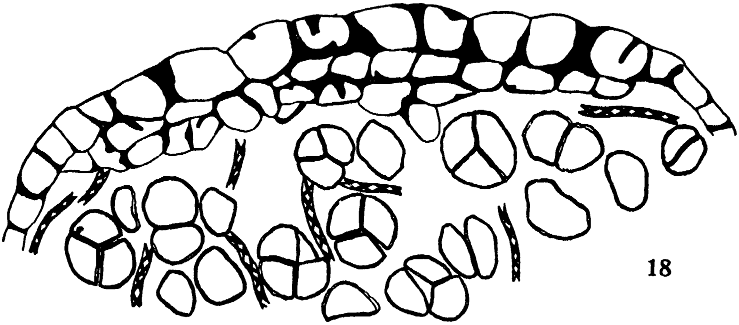
FIG. 16.—Great increase in size of capsular region; spore mother cells with heavy walls in rows; narrow elaters between.

mother cells. In the meantime a new cell wall has been forming around each spore mother cell (fig. 22). This new wall, although it never becomes thick like the old wall which has dissolved, is very definite. The spore mother cells remain in the original rows until after nuclear divisions have occurred within them, being held there apparently by traces of the old dissolved walls. Each spore mother cell, having increased somewhat in size, now divides to form a tetrad of spores. These spores develop the characteristic



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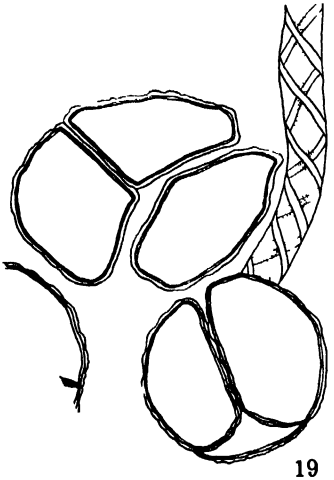

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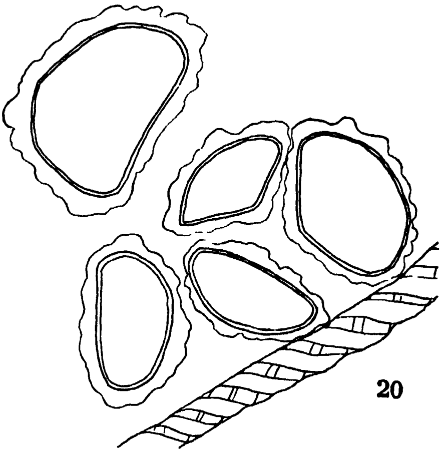

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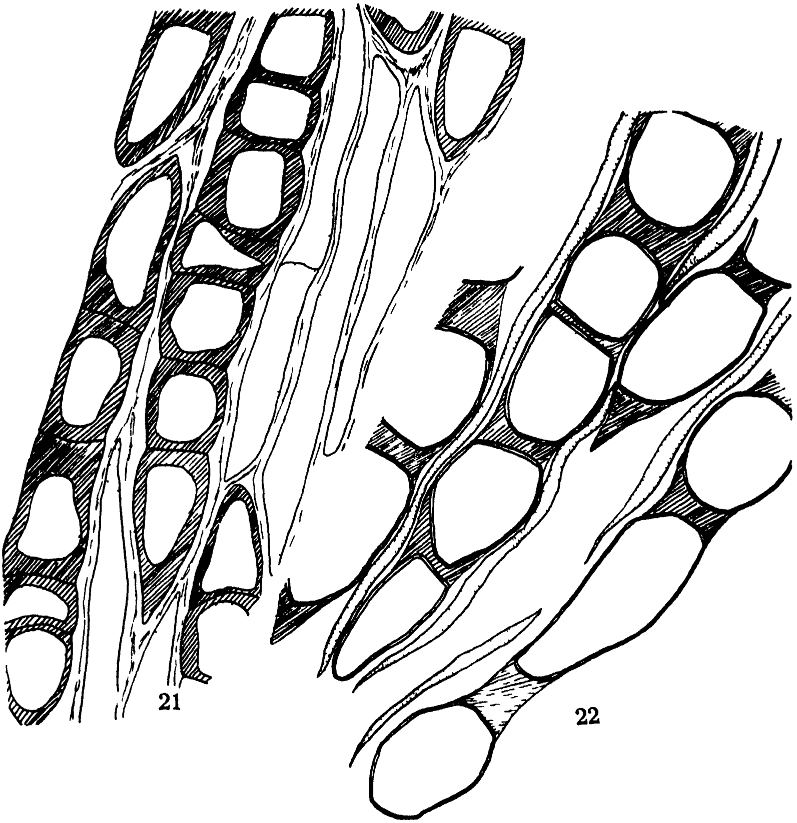
Figs. 18–20.—Fig. 18, apex of almost mature sporophyte, showing thickenings on walls of cells of sterile cap; fig. 19, spores still in tetrads, with walls newly forming; cytoplasm still present in elaters in form of narrow strand down center; thickenings of elaters rather narrow; fig. 20, spores mature with thickened walls; elaters mature with broad spiral band thickenings and no cytoplasmic content.

walls consisting of three layers, endospore, exospore, and irregular epispore. The spores remain together in tetrad formation until their walls are well formed (figs. 18, 19).

Not until nuclear division has occurred within the spore mother cells do the elaters begin to develop their characteristic thickenings. Up to this time the cell wall of each elater has been thin and its contents have been faintly granular. As the thickenings (spiral bands) begin to appear, the cytoplasm seems to lie in a strand running longitudinally through the center of the cell (fig. 19). The cytoplasm gradually disappears, and at the same time the thickenings of the wall become wider and more definite. The end result, at the time when the spores separate from the tetrad, is a very long, apparently empty cell with two spiral band thickenings on the inner side of the wall (fig. 20). These spiral bands run in opposite directions, so that they cross each other at regular intervals, giving a braided effect when looked at superficially.

At about the time of completion of the nuclear divisions in the spore mother cells, the first indications of thickening on the walls of the cells of the capsule wall begin to appear. These thickenings continue to grow until they form narrow girdling bars, which almost completely encircle the elongated cells of the capsule wall. The number of these thickenings in a given cell varies, four or five being the average. At the apex of the capsule they are very wide and heavy. Not only do they occur in the cells of the capsule wall, but also in those of the apical group of sterile cells within the capsule wall (fig. 18). This small group of cells, consisting of two or three layers of cells, has persisted since the time of their first differentiation (fig. 11). The thickenings in this region are not as heavy, nor do they encircle the cell in as regular fashion, as those in the cells of the capsule wall. Often they are in the form of short bars, tapering at each end. Thickenings of the walls also occur in the layer of cells at the base of the capsule wall. This irregularity in appearance is probably due partly to the fact that the cells of this layer are not as consistent and regular in shape as those of the outer capsule wall. It is also due to the form of the thickenings themselves, for, when compared with the thickenings of the capsule wall cells, they are narrower, not so long, and occur less frequently in a given cell.

While the sporogenous cells are dividing and elongating, the cells of the foot and seta are also dividing. The cells of the lowest layer of the foot are large, have large nuclei, and at a later period take a dense stain, in accordance with the usual condition of such haustorial cells. In the majority of cases the foot as a whole is rather globular in shape, and consists of a few cells.



FIGS. 21, 22.—Fig. 21, spore mother cells in rows with very heavy walls; fig. 22, new walls being formed around spore mother cells; old walls gradually disappearing; narrow elaters occurring between rows of spore mother cells.

At the time of the first elongation of the sporogenous cells, divisions occur in the region of the seta so that the sporophyte from this time on is longer than broad (fig. 12). Divisions within this seta region are relatively few for some time, being just sufficient to keep pace with the general increase in size of the whole sporophyte. But at the time when the spores are separating from one another in the tetrads, many transverse divisions occur in the cells of the region of the seta (fig. 17). As a result of the elongation of these newly formed cells the capsule, at complete maturity, will be pushed out through the surrounding calyptra and perianth.

During the development of the sporophyte, the cells of the venter of the archegone have divided, keeping pace with the growing embryo. This division of cells is begun immediately after fertilization has occurred and continues until the spores are formed, thus forming the calyptra. At the same time divisions have occurred in the tissue below the base of the archegone, resulting in the formation of a broad massive cushion, which pushes the developing sporophyte farther out from the archegonial head. As this division begins, a collar of cells at the base of the archegone begins to divide, forming a single-layered sheath of cells, the perianth. Just as in *Marchantia polymorpha*, the developing sporophyte is then surrounded by the old wall of the archegone, the calyptra, a sheath, the perianth, and the involucre, which incloses the whole group of archegonia and developing sporophytes beneath each lobe.

Discussion

Following the first transverse division of the embryo, the formation of vertical walls to form quadrants is not observed in *Marchantia chenopoda*. In a few cases in later embryos there are some indications that the original divisions formed quadrants of this nature; but on the other hand there are many embryos in which there is no possible suggestion of a quadrant or octant division.

The division planes which are conspicuous are those of two transverse divisions, one being the first division of the zygote, the other a division of either the hypobasal or the epibasal cell. These divisions remain conspicuous for some time. The lower one, which may be observed at a very late period, appears to separate the foot from the rest of the sporophyte. If one may assume that a wall which is continuous and is somewhat heavier than other walls is the first division wall, then this wall which marks off the foot from the region of the seta and capsule is the first division wall. Whether the capsule and seta are separated from each other by the second transverse division is not to be so easily demonstrated in the later stages. In the earlier stages, however, there is considerable evidence that this is the case.

To return to the three regions, basal, middle, and apical, as divisions occur in these regions and there appears a difference in the staining reaction (fig. 7), it is to be observed that the cells of the basal region are most lightly stained, those of the middle come next in density, and those of the apical region are the most dense. The apical and middle regions are to be distinguished not only by the continuous cell wall, separating them, but also by a difference in cytoplasmic content. As divisions continue and density increases, the central cells of the apical region, destined to become the sporogenous tissue, are the most dense; those at the periphery of the apical region, which are to give rise to the capsule wall, are a little less dense; and the middle and basal regions show about the same ratio in staining capacity to one another as previously, both regions being lighter than the apical region.

As the embryo increases in size and the sporogenous cells become more dense in cytoplasmic content, the division wall which marks off the foot remains conspicuous. Between this line and the sporogenous region there is a region which corresponds favorably with the earlier middle region, and also with the seta of later stages (figs. 9, 10). As these regions are followed in the succeeding development, it is evident that the sporogenous region, with its outer row of cells destined to become the capsule wall, develops into the capsule; that the lower part, the earlier basal region so clearly marked off by the early division wall, becomes the mature foot; and that the part between, the earlier middle region, marked off definitely from the foot and somewhat vaguely from the capsular region, is to become the seta.

The history thus outlined does not agree with that reported for *Marchantia polymorpha* (10), nor with that in many of the other Marchantiales, in which the first divisions result in quadrants. This quadrant type of embryo has been reported for *M. polymorpha* by DURAND (10). It is also reported for *Riccia*, *Targionia*, and *Fim-*

briaria californica by CAMPBELL (3), for *Corsinia* by MEYER (18), for *Cyathodium foetidissimus* by LANG (16), for *Cryptomitrium* by ABRAMS (1), and for *Conocephalum* by CAVERS (4).

The embryo of *Reboulia haemisphaerica* suggests in some ways that of *Marchantia chenopoda*, in that it is at first a filament of three or four cells (9, 13, 22). Such an embryo is also described for *Plagiochasma* by STARR (20), for a species of *Targionia* by O'KEEFE (19), and for *Geothallus* and *Sphaerocarpos* by CAMPBELL (3). In *Conocephalum*, according to CAVERS (4), a second transverse wall sometimes appears in the epibasal cell, although the usual condition is for vertical walls to divide the embryo into octants. GARBER (12) found a similar condition in *Ricciocarpus*, in which the usual occurrence was that of the octant form, but occasionally a row of three cells was observed.

In *Marchantia chenopoda*, it appears that after the first division, which forms the hypobasal and epibasal cells, another transverse division occurs in one of these. Whether this division is in the hypobasal cell has not yet been demonstrated conclusively, since no mitotic figures have been observed. It has been reported by DURAND (10) for *M. polymorpha* and by KIENITZ-GERLOFF (14) for *Preissia* that the hypobasal cell develops into the foot and seta, and the epibasal cell develops into the capsule. In *M. polymorpha* this decision is based apparently on the presence of a conspicuous transverse division between the regions of the seta and the capsule, and upon the fact that the staining reaction is such in the early stages that it appears that one half becomes capsule and the other half becomes foot and seta. On this same basis, it is to be noted in *M. chenopoda* that the conspicuous division wall (first?) is the one marking off the foot from the seta; and that at an early age the sporogenous, heavier staining region occupies not one-half, but approximately one-third of the young sporophyte.

This last point would tend to suggest that there might be a difference in the early embryogeny of the sporophytes of these two forms, for at the time when the sporophyte of *M. polymorpha* is still distinctly spherical in form, the sporophyte of *M. chenopoda* has elongated parallel to the long axis of the archegone. This elongation is apparently due to division and growth in the middle or seta region.

The larger number of cells in the region of the seta, as compared with *M. polymorpha*, is a difference which persists until the time when the true sporogenous cells and elaters are first to be differentiated in the capsular region. As a result, during this period of time, the sporophyte of *M. polymorpha* is broader than it is long, while that of *M. chenopoda* is longer than it is broad.

It is very important that it be noted that the sequence of divisions and development just outlined is not the one and only occurrence. Frequently the first divisions are extremely oblique. In such instances the differentiation of tissues occurs in the same general region (in relation to the position in the archegonium), but it does not follow the first division walls. DURAND makes clear that this also occurs in *M. polymorpha*. But when the walls are not too extremely oblique the succeeding divisions and development follow the description already given.

The occurrence of sterile cells at the apex of the capsule is a condition not reported by DURAND for *Marchantia polymorpha*. MEYER (18) reports such a sterile region, however, and CRIBBS (6), who also found a columella in one case, reports a sterile cap of cells in *M. polymorpha*. There is no indication of a columella in *M. chenopoda*. The sterile cap, consisting of two or three layers of cells, appears at a time when the cells of the sporogenous region are first beginning to elongate. It remains intact, increasing slightly in size, with the increase in size of the whole sporophyte. In the mature sporophyte the cell walls of this region become thickened in a manner similar to that characterizing the cells of the capsule wall.

This condition suggests that of *Conocephalum*, *Lunularia*, and *Dumortiera*, in which, according to CAVERS (5), there is a "well-developed apical thickening which is thrown off as a lid." In these forms the lower portion of the capsule splits into four to eight teeth, after the lid has fallen off. The dehiscence of the capsule of *Marchantia chenopoda* has not been observed. Such a thickened apical cap is also reported by HAUPT (13) for *Reboulia haemisphaerica* and *Preissia quadrata*. For *Marchantia*, CAVERS (5) reports that the apical cap is only indicated by "an imperfect or loose layer of cells lying within the normally single-layered capsule wall at the apex, and the capsule opens by teeth extending to the apex."

The foot presents another variation from *Marchantia polymorpha*. In *M. chenopoda*, in the majority of cases, it is bulbous in shape and consists of a few cells. In *M. polymorpha* the foot is "anchor-shaped," and usually made up of more cells. Occasionally, however, there appears in *M. chenopoda* a foot which shows a slight tendency to be somewhat anchor-shaped, but this is never as marked as in *M. polymorpha*. It seems that this occasional appearance of a differently shaped foot must indicate a condition that is not fixed; that, rather, there is here expressed a tendency to reduction in the foot, the haustorial activity being carried on by a few large cells, rather than by many smaller ones. The similar bulbous foot found in *Reboulia* by HAUPT is considered by him as one of the primitive features of the genus. There is the possibility that this is the condition in *M. chenopoda*. It seems strange, however, that in both *Reboulia* and *M. chenopoda* the advanced condition of a thickened sterile cap at the apex should be combined with a primitive condition in the foot. Because of this fact there is a tendency to conclude that the smaller foot of *M. chenopoda* indicates a reduction, rather than a primitive condition.

When the cells of the sporogenous region are well elongated, many of them are observed to be binucleate and sometimes multinucleate. This condition persists for some time, but when the cells become well separated from each other, and the elaters and true sporogenous cells can be distinguished because of the difference in size, all the cells appear to be uninucleate. It is probable that the binucleate condition is one which exists only until the ultimate division forming the elaters and the sporogenous cells, each of which will form a single row of spore mother cells.

Summary

1. The first transverse division of the zygote in *Marchantia chenopoda* separates the foot from the seta and capsule; that is, the foot develops from the hypobasal cell, the seta and capsule from the epibasal cell.

2. Two primary transverse divisions seem to be the regular occurrence in the embryo, instead of a quadrant division. These divide the embryo into apical, middle, and basal regions. The basal region

develops into the foot, the middle into the seta, and the apical into the capsule.

3. The sporophyte, soon after the first difference in staining is observed, becomes longer than it is broad. This is due to divisions in the region of the seta. In *M. polymorpha* the cells of the stalk remain relatively few in number, while the rest of the sporophyte goes on dividing, the result being that during the same period of development as in *M. chenopoda* the sporophyte is broader than it is long.

4. The sterile cap of cells present at an early stage in the capsule persists throughout the life of the sporophyte; the walls of these cells, when mature, taking on thickenings similar to those of the cells of the capsule wall.

5. The cells which are to form the layer of cells across the base of the capsule are distinguished by their staining reactions at the time of the first differentiation of the capsule wall cells from the sporogenous cells.

6. Many of the cells of the sporogenous region, during early elongation, are binucleate.

7. The foot is bulbous in shape and occasionally shows a slight tendency to be "anchor-shaped." Such a condition probably indicates a reduction in this region.

8. The antherid and archegone show the same fundamental structure and development as in *M. polymorpha*.

The writer wishes to express grateful appreciation to Professor GEORGE S. BRYAN for his advice and encouragement at the beginning of the study, and to Professor C. E. ALLEN for his suggestions and careful criticism.

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MORPHOLOGICAL STUDIES ON A NEW SPECIES OF MARCHANTIA

ENID A. HEBERLEIN

(WITH TWENTY-ONE FIGURES)

In this study the investigation has been concerned mainly with the development and structure of the sporophyte of a species of *Marchantia* which is as yet undescribed. The development of the sex organs has also been traced. The history of the development of the sporophyte, and to some extent that of the sex organs, is followed and compared with the corresponding history for *M. polymorpha.*

Materials and methods

Material of this species was collected by Professor GEORGE S. BRYAN in several localities in the province of Huanuco in Peru. The particular material used in the present study was found at Cani, a village about 18 miles east of the town of Huanuco, the capital of the province.

The plant looks much like *Marchantia polymorpha*, with its flat thallus and dichotomous branching. The successive forks in the branching are usually 1.5–2.5 cm. apart. The margin is entire or slightly undulate. Ventral scales are in two distinct rows on the median riblike portions. Their shape is spatulate. The male receptacle is borne on a stalk 1–1.5 cm. high, and when mature is 3 cm. broad. There are five to eight rays in palmate arrangement, the basal sinus being almost a straight line.

The female receptacle is borne on a stalk 3 cm. long, and at maturity is 5–7 mm. broad. There are four to eight rays which are so poorly defined as to be almost indistinguishable. The general outline when mature is that of a skull cap. Gemmae cups are numerous.

The material was killed in the field at various times during the months of April, May, and June, 1923. The killing fluid used was prepared according to the following formula: 1 cc. of glacial acetic acid, 1 gm. of chromic acid, and 400 cc. of water.

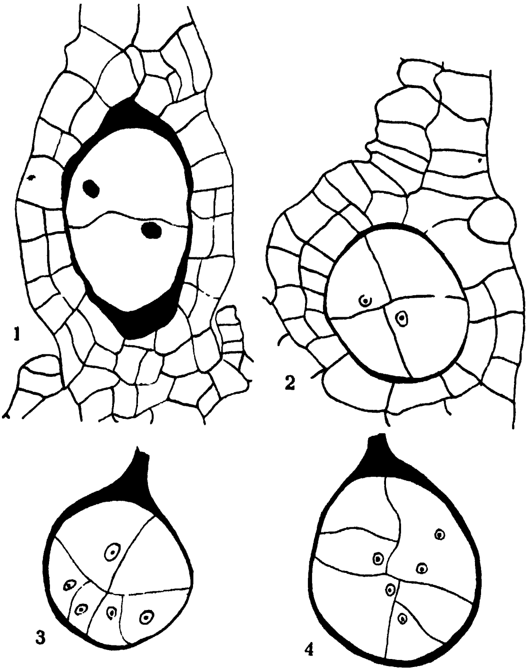
After eight months the material was removed from the fixing fluid, washed, and run through a close series of alcohols to 70 per cent. At this stage it was turned over to the writer. It was fully dehydrated and then cleared in an alcohol-chloroform series to pure chloroform. Then it was imbedded in paraffin, sectioned at thicknesses of 5 to 10 μ, and the sections stained with safranin in combination with light green.

Development of sporophyte

The first division in the zygote is usually transverse (figs. 1, 3), or in some cases is somewhat oblique (figs. 2, 4). This first division wall is apparent later, and may be traced for several stages in the sporophyte. The plane of the second division is almost perpendicular to that of the first, dividing the embryo into quadrants (fig. 2). At this time the embryo broadens out, becoming spherical or somewhat ovoid. The next divisions vary in different embryos. In fig. 3 the two lower quadrants are divided by anticlinal walls, while in fig. 4 the divisions following the second are in diagonally opposite quadrants. If the embryo has broadened out considerably, the corresponding division is in a plane parallel to that of the first vertical division (fig. 5). A few more anticlinal divisions occur, and then periclinal walls begin to be formed (fig. 6). Further divisions are periclinal and anticlinal without definite sequence, until a spherical mass of cells is produced in which the primary division walls can be recognized clearly (figs. 7, 8). The embryo has increased but little in size up to this time, so that its component cells become successively smaller as division progresses.

While this development is proceeding in the embryo, changes are taking place in the venter of the archegonium and in the tissue at its base. By transverse divisions this basal tissue grows out rapidly until it forms a tubular sheath, the pseudo-perianth, surrounding the venter. This is a single layer of cells in thickness. The beginning of this sheath is shown in fig. 1. Another change is noted in the wall of the venter and in the base of the neck. Periclinal divisions occur until two or three layers of cells are formed around the young embryo; the calyptra thus formed serves as a protective covering for the growing embryo (figs. 1, 5).

The embryo and surrounding tissues now enlarge rapidly, the embryo being for a time nearly spherical (fig. 9), and later broader than long (fig. 10). The first indication of a differentiation within the embryo sporophyte is a change in the staining capacity of the cells. Those in the distal half, which are to form the capsule, become richer in protoplasmic content, while those of the proximal half (the future seta and foot) take less stain (fig. 9). The two portions thus differentiated are usually separated by the first transverse division wall. This is very plain in fig. 9. In fig. 11 there is in part more than



Figs. 1–4.[1]—Fig. 1, transverse division of zygote and beginning of pseudo-perianth; fig. 2, embryo divided into quadrants, periclinal divisions in venter wall; fig. 3, one type of further development of embryo after quadrant; fig. 4, another type of development following quadrant stage.

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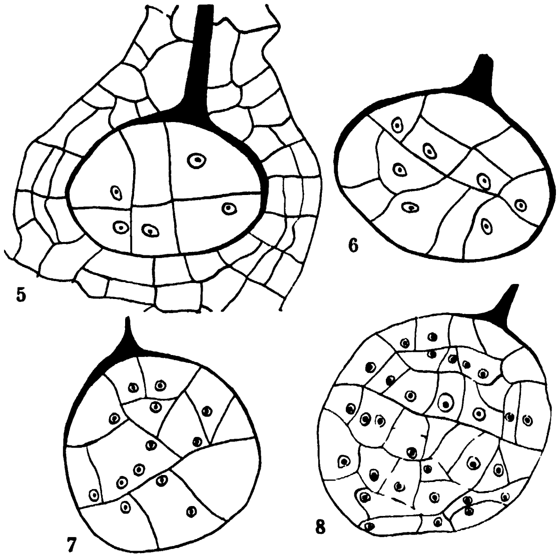
[1] All figures were drawn with the aid of a camera lucida at table level, and show the following magnifications: figs. 1–11 ×700; fig. 12 ×555; fig. 13 ×325; fig. 14 ×250; figs. 15, 17, 20 ×1150; fig. 16 ×100; fig. 18 ×120; fig. 19 ×140; figs. 21, 22 ×690.

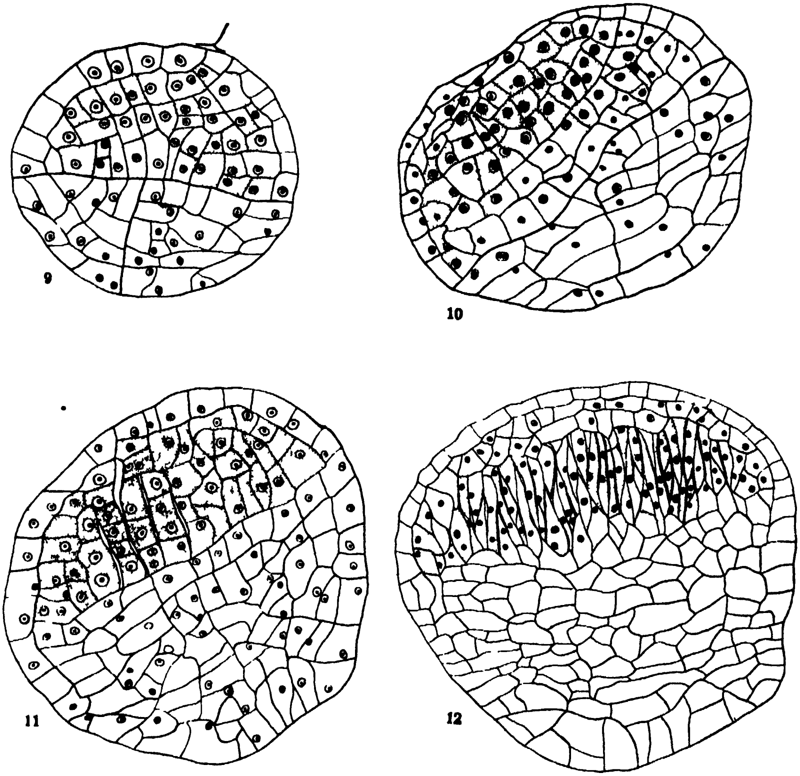
one layer of cells below the potential sporogenous tissue which does not take deep stain, but is very similar to the single peripheral layer of cells in the upper part of the capsule, which cells will become the outer sterile wall of the capsule. It is likely that these cells not only form the inner boundary of the capsule, but may also contribute to some extent in the formation of the stalk.



FIGS. 5–8.—Fig. 5, third type of development following quadrant, further divisions in venter wall; fig. 6, first periclinal walls appearing; figs. 7, 8, further periclinal and anticlinal walls.

The cells of the sporogenous tissue are at first irregularly isodiametric and similar to the cells of the seta (fig. 10). Then they elongate somewhat in a direction parallel to the axis of the archegonium, and there is a tendency in the rows of elongated cells to separate slightly from one another (fig. 11). A slight bulging of the basal cells of the embryo indicates the beginning of the foot.

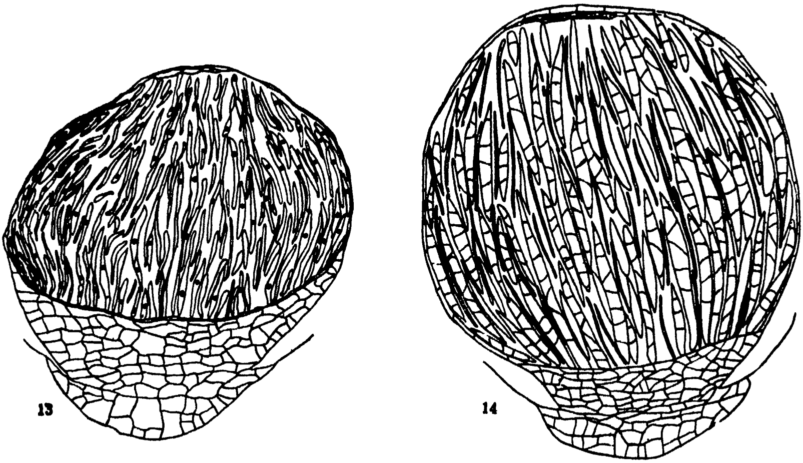
In fig. 12 a more advanced condition is shown. The foot has become a little more prominent, but has not yet assumed its final shape. The capsule portion is broad and the potential sporogenous cells are numerous, plainly elongated and often binucleate. At this stage also a cap of cells, destined to remain sterile, can be observed in the apical region just beneath the capsule wall. The cells of this cap are approximately isodiametric, thus resembling the sporogenous cells at an earlier stage, and are less deeply stained than the elongated sporogenous cells.



FIGS. 9–12.—Fig. 9, first differentiation of sporogenous tissue more deeply stained; fig. 10, sporophyte broader than long; fig. 11, beginning of separation longitudinally of sporogenous cells; fig. 12, further separation (note binucleate cells and sterile cap).

In a still older sporophyte (fig. 13) the foot has penetrated more deeply into the tissue at the base of the archegonium, and expanded laterally, forming an anchor-shaped absorbing organ whose cells are filled with deeply staining material. The cells of the sporogenous tissue have separated completely from one another and have elongated still further. The elongated cells are now uninucleate; evidently transverse cell divisions have occurred since the stage of fig. 12.

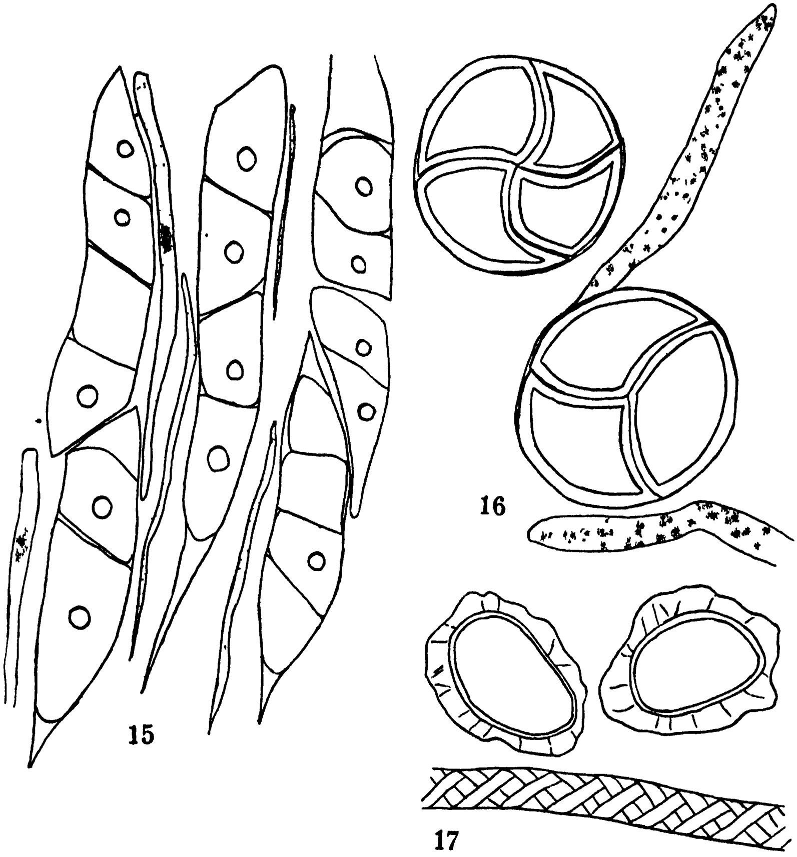
A few of these elongated cells are narrower than the others, and this is the first indication of a differentiation between sporogenous cells and elaters. At this stage, however, the difference is barely noticeable. At a somewhat later stage (fig. 14) the distinction between sporogenous cells and elaters is much more marked, the sporogenous cells having become larger and the elaters remaining comparatively very slender. The two kinds of cells alternate irregularly. The cap of sterile cells is still visible in the stages shown in both fig. 13 and fig. 14. The cells composing it have become much flattened against the capsule wall and seem shrunken. At this time the seta is very short and its cells are broad. The capsule has become much larger in proportion to the stalk and foot regions, and is almost spherical in outline.



FIGS. 13, 14.—Fig. 13, sterile cap still present, elaters more slender than sporogenous cells; fig. 14, rows of spore mother cells alternating irregularly with elaters, sterile cap.

The sporogenous cells, after increasing in size, become divided by transverse walls to form rows of two to eight cells each, all still inclosed by the old wall of the elongated cell (fig. 15). The cells composing these rows are the spore mother cells. They are irregular in shape, being flattened against the old walls of the mother cells and also where they are contiguous with one another. Each of these spore mother cells becomes rounded and later divides to form four spores.

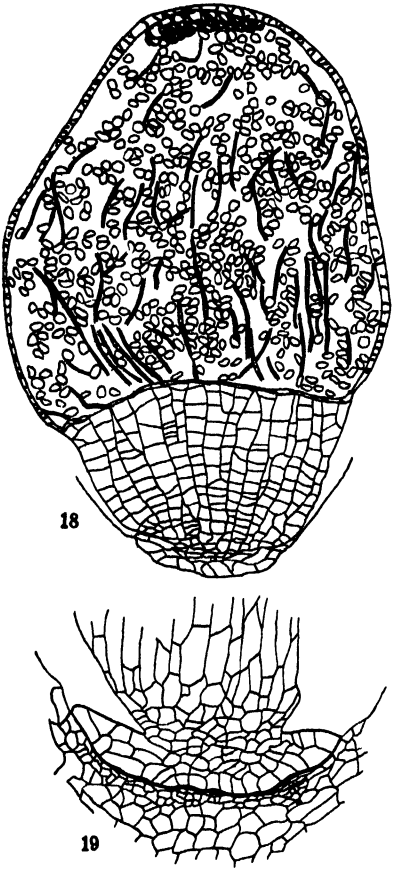
These spores remain in tetrads for a time (fig. 16). The elaters at this time are still granular in content, but the granules have become aggregated into groups. By the time the spores are mature and have separated from their tetrad grouping the elaters have matured, having formed spiral bands on the inside of the wall (fig. 17).



FIGS. 15–17.—Fig. 15, detailed drawing showing rows of spore mother cells and slender elongated cells, young elaters; fig. 16, tetrads of spores derived from spore mother cells and elaters with walls still unthickened; fig. 17, mature spores and elater.

The nature of the material prevented full working out of the detail of the development of elaters. They seem to have developed from elongated cells among the sporogenous cells as already stated.

The contents are at first uniformly granular; then the granules aggregate, and later become empty cells in which some material, possibly the granules, has been deposited in two spiral bands which are regular and uniform, running the length of the long elater.



FIGS. 18, 19.—Fig. 18, details of mature sporophyte; fig. 19, detail of foot of mature sporophyte.

Fig. 18 shows a mature sporophyte. At this time the foot is small in proportion, still keeping its anchor shape; however, the cells of the seta have grown and divided, so that the capsule has been pushed up through the calyptra, and there is a marked thickening on the walls of the sterile cells of the capsule wall and of the cap of the capsule. The only other change in the sporophyte after this stage is an elongation of the much divided cells of the seta (fig. 19), so that the capsule is pushed out still farther.

Sporophyte

The position of the first division wall in the zygote varies within narrow limits. It may be transverse; in fact, in all the preparations in which the first division only had occurred the plane was horizontal. But to judge from some later stages of the embryo, this first division may be obliquely transverse (figs. 2, 4, 6). This first division wall may be followed through the development of the embryo to a stage as late as that of fig. 11, and, in general, this wall seems to divide the sporophyte into a distal portion which will become the capsule, and a proximal portion which gives rise to the seta and foot. This is usually the case in *Marchantia polymorpha* also (4).

Succeeding divisions form quadrants, and then anticlinal and periclinal walls form a mass of cells which is almost spherical. As in *M. polymorpha* (4), there is an increase in size; then a stage in which the embryo is broader than long (fig. 10); and still later it becomes longer than broad (figs. 13, 14). The last mentioned growth in length does not occur until the sporogenous cells have elongated considerably; thus it is the capsular region which at first increases in length. Later the seta elongates, and the sporophyte becomes much longer than broad.

The sporogenous tissue is first differentiated by a change in staining capacity. The isodiametric cells of this distal region take a heavy stain. A single peripheral layer of cells in the capsule portion takes a lighter stain. The other cells elongate, often become binucleate (figs. 11, 12), and later by divisions become uninucleate again. These elongated, heavily staining cells are the ones from which elaters and the spore mother cells will arise. There seems to be further elongation, and then the elaters remain slender while the other, now broader, cells divide transversely to form each from two to eight spore mother cells. DURAND (4) has not followed this part of the history in much detail, but says that the long sporogenous cells may be divided by transverse or by transverse and longitudinal walls into groups of eight, rarely four, cells. Thus there may be formed in *M. polymorpha* biseriate or uniseriate rows of spore mother cells. It may be noted that only transverse divisions are observed in the species under study here. As in *M. polymorpha*, the spore mother cells divide, each to form four spores which remain in the tetrad arrangement for a time. During this time the elaters have been developing from slender elongated cells to mature form with spirally thickened walls, but the process of tracing this development met with difficulty and the exact history in detail is lacking.

There is a sterile cap of cells just beneath the capsule wall in the apical region. This cap of two or three layers of cells appears at a time when the cells of the sporogenous region first elongate, is present but greatly compressed at the time the spore mother cells are formed but still arranged in rows (fig. 14), and is still to be seen in the mature sporophyte, where the walls of the cells composing it are thickened spirally and with bands as are the cells composing the sterile wall of the capsule (fig. 18). DURAND does not report such a cap. CRIBBS (2) did find a sterile region of cells in *M. polymorpha.* CAVERS (1) reports that in *M. polymorpha* "the apical cap is only indicated by an imperfect or loose layer of cells lying within the normally single-layered capsule wall at the apex."

In *M. polymorpha,* as described by DURAND, the foot becomes anchor-shaped. The cells of the seta are in quite regular rows; they are at first broader than long and later become much elongated (fig. 19). Since the capsule is fully mature and the stalk is still short (fig. 18), it is probable that the elongation of the stalk is coincident with the dehiscence of the capsule. Whenever a long stalk is found the capsule has discharged its spores and is losing its shape.

Sex organs

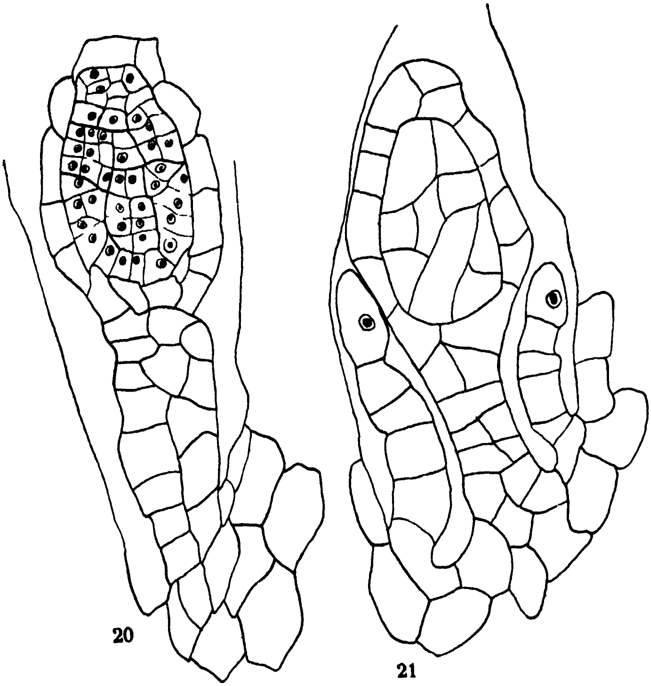
During this study it has been found that in this as yet unnamed species of *Marchantia* the development of the antheridium follows in general the corresponding history in *M. polymorpha.* The points of difference noted were the length and size of the stalk and the presence of what may be called paraphyses (6). In the species under investigation the stalk has four tiers of large cells while the stalk of *M. polymorpha* consists of two small cells in approximately similar stages in development. The divisions in the stalk cells occur later in *M. polymorpha,* and ultimately there is formed a stalk of five or six tiers of cells. The stalk in this unnamed species finally becomes six or seven cells long and quite massive, and it is noticeable that the character of relatively larger size is early manifest (figs. 20, 21).

The other noticeable feature was the outgrowth at the base of the young antheridium which elongated and in some cases became two- or three-celled. These paraphyses grow up around the organ in its cavity (figs. 20, 21). In later stages only traces of such cells are found and their function is unknown (6).

The development of the archegonium is very regular, and compares almost exactly with that of *M. polymorpha.* There are usually four neck canal cells, but five are not so uncommon in this species as in *M. polymorpha.*

ANDROGYNOUS RECEPTACLES

During the sectioning of this material two androgynous receptacles were discovered. These had much the shape of the female receptacles. The archegonia were borne on the under side as they ordinarily are, and the antheridia were in cavities opening to the upper side. CUTTING (3) found some androgynous receptacles on a hybrid species of *Marchantia;* but in that instance the antheridia were borne on proliferations from the under side of the receptacle, while the archegonia were in the ordinary position. The occurrence of androgynous receptacles in this new species may indicate a tendency toward a monoecious condition. There was nothing abnormal in the appearance of the receptacles or in the number or arrangement of the sex organs.



FIGS. 20, 21.—Fig. 20, maturing antheridium with long stalk and long mucilaginous hairs; fig. 21, more mature stage of antheridium with massive stalk evident.

Summary

1. This study deals with an unnamed species of *Marchantia* growing in the Peruvian Andes. Male and female receptacles were studied and the development of the sporophyte and sex organs was traced.
2. The first division in the zygote is transverse or obliquely transverse, dividing the embryo into a distal portion which will become the capsule and a proximal region which will become foot and seta.
3. The second division is perpendicular to the first.
4. Periclinal and anticlinal divisions form a spherical mass of cells. The distal region takes a deeper strain and later forms sporogenous cells.
5. A peripheral layer of cells around the sporogenous cells forms the capsule wall; the walls of these cells later show bandlike thickenings.
6. A cap of sterile cells is present in the apical region from the time the cells of the sporogenous region first elongate until the sporophyte is mature. These cells also have thickenings.
7. At one stage the elongated sporogenous cells are binucleate, but by divisions soon result in uninucleate cells, and these give rise to rows of spore mother cells.
8. The stalk remains short until the spores are mature, and then the cells elongate and push the capsule out through the calyptra.
9. The antheridium has a typical development and also has paraphyses and a noticeably long and massive stalk.
10. The archegonium development is typical of *M. polymorpha*.
11. Two androgynous receptacles with both kinds of sex organs in their ordinary positions were found.

The writer wishes to express grateful appreciation to Professors George S. Bryan and C. E. Allen for suggestions and criticisms in the progress of this study.

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COMPARATIVE EFFECT OF TEMPERATURE ON RATE OF PURE CHEMICAL REACTIONS AND RATE OF SUGAR UTILIZATION BY A PLANT AND A COLD BLOODED ANIMAL

G. C Wickwire, L. D. Seager, and W. E. Burge

(WITH TWO FIGURES)

It is recognized that temperature influences the rate of chemical reactions, a rise in temperature increasing the rate and a fall in temperature decreasing it. A number of investigators have studied the effect of temperature on the rate of the chemical reactions of several different chemicals, and found an increase in rate varying anywhere from 1.2 times to as high as 3.6 times for a rise of each 10° C. This has been generalized by VAN'T HOFF (8) into the rule that for every rise of 10° C. the rate of reaction is about doubled or trebled. It is also recognized that temperature influences the rate of physiological processes in plants and animals. It is known that an increase in light intensity increases photosynthesis, and a rise in temperature increases carbon dioxide assimilation, oxygen elimination and growth in plants and a fall in temperature decrease these processes with resulting dormancy. Some investigators (1, 5–7) claim that the temperature relations of these processes are such that they conform to the VAN'T HOFF rule for pure chemical reactions, while others (2–4) claim that they do not.

One of the earliest divisions of the animal kingdom into cold blooded, warm blooded, and hibernating animals, was made on the basis of temperature. It is known that oxygen absorption, carbon dioxide elimination, heat production, and hence oxidation is increased by a rise in temperature and decreased by a fall in temperature in cold blooded animals, resulting in a fall in body temperature and dormancy. Low temperature, on the other hand, increases oxygen absorption, carbon dioxide elimination, heat production, and hence oxidation in warm blooded animals, and is one of the factors responsible for the maintenance of the constant body temperature of these animals.

The object of this investigation was to determine the effect of various temperatures on the rate of sugar utilization by the plant *Spirogyra porticallis*, and by a cold blooded animal, the ordinary gold fish, in order to determine how closely the effect of temperature on sugar utilization coincided with its effect on pure chemical reactions.

The respiratory quotient is the index usually used to the amount of sugar metabolized, a rise in the quotient indicating an increase in sugar metabolism, and a fall, a decrease. In this investigation sugar utilization, as well as the effect of various temperatures on the rate of this utilization, was determined directly according to the following procedure.

Eight hundred cc. of 0.1 per cent dextrose solution was prepared and divided equally into eight beakers of 100 cc. each. Two gold fish of approximately the same size and with a combined weight of approximately 5 gm. were then introduced into each beaker. Air was bubbled through the sugar solutions to insure an adequate supply of oxygen to the fish. The beakers were then placed in water baths at 5°, 10°, 15°, 20°, 25°, and 30° C. respectively. A small amount of sugar solution was removed immediately from each of the beakers and sugar determinations were made according to the method of BENEDICT. At the end of the experiment, which lasted 24 hours, sugar determinations were made again. The results of the average of five series of such experiments are shown in fig. 1. It will be seen that the fish kept at 5° C. used 15 per cent of the sugar in 24 hours, and those kept at 10°, 15°, 20°, 25°, and 30° C. used 24, 30, 32, 35, and 46 per cent of the sugar respectively. By comparing these figures it will be seen that sugar utilization was decreased at the lower temperatures and increased at the higher. The effect on the sugar metabolism of the fish at 0° C. is not given in the chart, because it was found in most of the experiments that the fish died at this low temperature. It was not possible to keep the temperatures of the different baths absolutely constant as given in the chart; however, they were kept within less than 1° C. of the temperatures indicated.

The method of procedure in determining the effect of various temperatures on the rate of sugar utilization by *Spirogyra* was as follows. A considerable amount of *Spirogyra* was collected from a nearby stream, brought to the laboratory, and the excess of water removed by gently squeezing with the hands. This material was then divided into smaller portions of 40 gm. each. Seven of these portions were introduced into 200 cc. of 0.1 per cent dextrose solutions in flat bottomed dishes 16 cm. in diameter. The dishes were then introduced into water baths at 0°, 5°, 10°, 15°, 20°, 25°, and 30° C. respectively and kept for 30 hours. A small quantity of sugar solution was removed immediately from each of the dishes and sugar determinations were made according to the method of BENEDICT. Sugar determinations were also made after 30 hours, at the end of the experiment. The results of the average of five series of experiments are shown in fig. 1. It will be seen that the *Spirogyra* kept at 0° C. for 30 hours used 4 per cent of the sugar; that kept at 5° C. used 7 per cent; and that kept at 10°, 15°, 20°, 25°, and 30° C. used

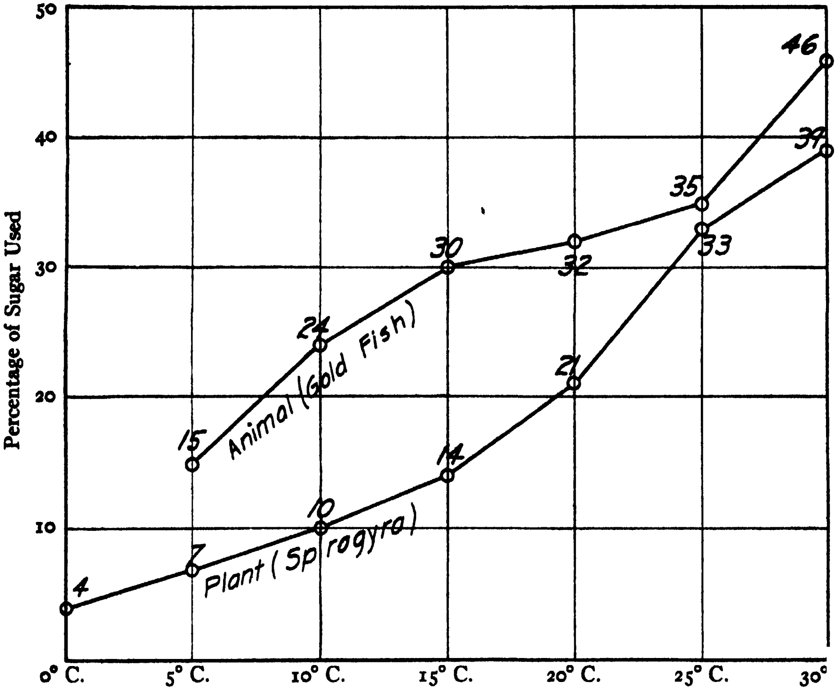

FIG. 1.—Curves showing that sugar utilization by gold fish and *Spirogyra* are decreased by low temperatures and increased by higher temperatures.

10, 14, 21, 33, and 39 per cent of the sugar respectively. By comparing these figures it may be seen that sugar utilization by *Spirogyra* was decreased at the lower temperatures and increased at the higher temperatures, just as was found to be the case with the gold fish.

A comparison of the two curves in fig. 1 brings out certain facts regarding sugar utilization by the animal and the plant. In the first place, it will be seen that the utilization of sugar by the animal was greater at all temperatures than that by the plant, although the weight of the animal used was only 5 gm. and the weight of the plant used was 40 gm. This is in keeping with the fact that metabolism in the animal is more intense than it is in the plant. In the second place, lowering of the temperature produced a greater decrease in sugar utilization in the plant than it did in the animal. At 25° C., for example, the amounts of sugar used by the plant and animal were almost the same, namely, 33 and 35 per cent respectively; while at a low temperature, 5° C., the amount of sugar used by the animal was more than twice as great as that used by the plant, namely 15 and 7 per cent respectively.

It was shown by VAN'T HOFF that if the logarithm of the reaction rate be plotted against the reciprocal of the absolute temperature at which the reaction takes place, a straight line is obtained in case of pure chemical reactions. The object was to plot curves, using the data given in fig. 1, for the effect of temperature on the rate of sugar utilization by the gold fish and *Spirogyra*, and to determine how closely they approach a straight line.[1] In fig. 2 the straight unbroken line is the theoretical curve obtained if the effect of temperature on the rate of sugar utilization by *Spirogyra* had been the same as it is on pure chemical reactions; while the broken line curve is constructed with the use of the data actually obtained from the effect of temperature on sugar utilization by *Spirogyra*. It may be seen that the broken line curve approaches very nearly the unbroken straight line curve, except at 0° C., and hence it may be concluded that the effect of temperature on the rate of sugar utilization by *Spirogyra* is the same as its effect on pure chemical reactions, except at this low temperature. The explanation that suggests itself for this exception is that the low temperature impaired the mechanism for normal physiological oxidation in the living plant. It is recognized that a relatively high temperature is required to bring about the oxidation of sugar in vitro, while in vivo this oxidation is brought about at the temperature of the living cell; hence there must be a mechanism whereby this oxidation can proceed at the temperature of the cell.

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[1] Thanks are expressed to Mr. D. F. BABCOCK of the Department of Physical Chemistry for the curves.

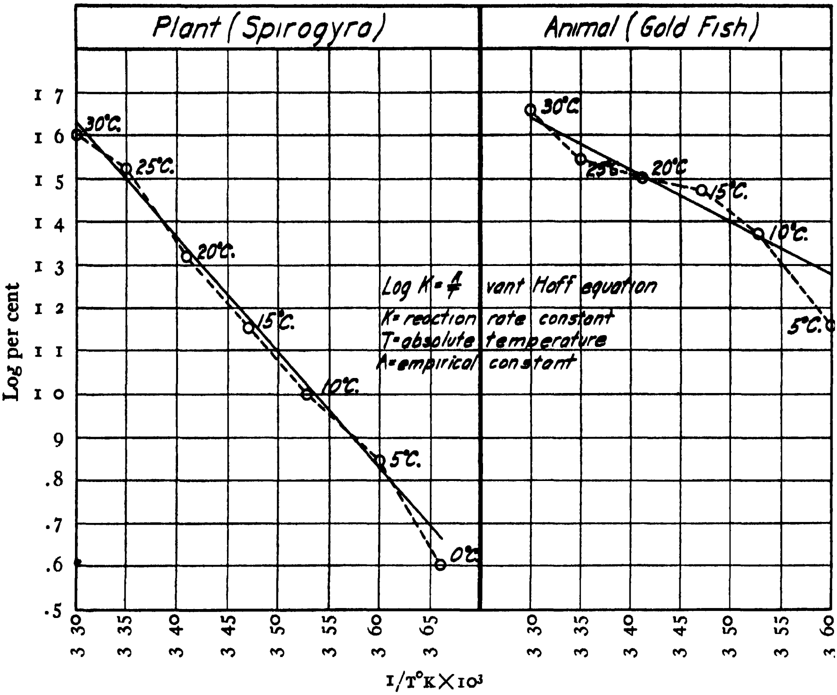

FIG 2 —Chart showing that broken line curves plotted with use of experimental data on effect of temperature on rate of sugar utilization by *Spirogyra* and gold fish with use of VAN'T HOFF equation approach rather closely to straight line curve for pure chemical reactions

Fig. 2 also shows the curve plotted with the use of the VAN'T HOFF equation for pure chemical reactions, using the data obtained in fig. 1 on the effect of different temperatures on the rate of sugar utilization by the gold fish. It will be seen that the broken line curve, which was constructed with the use of the data obtained with the gold fish, approximates roughly the straight unbroken line curve for pure chemical reactions, except at the temperature of 5° C. This approximation to a straight line, however, is not so close with the gold fish as it is with the *Spirogyra*. As with the *Spirogyra* so with the gold fish, the greatest deviation from the straight line curve, and hence from VAN'T HOFF's law for pure chemical reactions, occurs at the lowest temperatures used. The possible reason for this deviation at the low temperature may be again that the low temperature of 5° C. impaired the normal physiological mechanism for the oxidative processes of the fish.

The following precautions were taken and checks made in the preceding experiments. Air was bubbled through sugar solutions for 30 hours without the presence of fish or *Spirogyra*, and it was found that this had practically no effect on the sugar solutions. It was found that the sugar was used only when the fish or the *Spirogyra* was present, and that the utilization ceased upon the removal of either. From these observations it was concluded that the fish and the *Spirogyra* were responsible for the utilization of the sugar observed in these experiments.

Summary

1. Raising and lowering the temperature produced an increase and decrease in sugar utilization by the plant *Spirogyra*, and by a cold blooded animal, the ordinary gold fish, just as it does in pure chemical reactions.

2. Lowering the temperature produced a greater decrease in sugar utilization by *Spirogyra* than by the gold fish.

3. The effect of temperature on the rate of sugar utilization by *Spirogyra* and the gold fish followed very closely VAN'T HOFF's law for pure chemical reactions, except at very low temperatures. The explanation that suggests itself for this deviation from the law is that the normal physiological mechanism for sugar utilization in the living cells of the plant and animal is impaired by very low temperatures.

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OSMOTIC PRESSURE AND PH MEASUREMENTS ON CELL SAP OF PINUS PONDEROSA

FLOYD W. GAIL AND WM. H. CONE

(WITH TWO FIGURES)

Introduction

It has been found by the senior writer (1) that the osmotic pressure of expressed cell sap in some deciduous and non-deciduous plants reached a maximum during the winter months; that there was a decrease in the spring, and a gradual rise again with the coming of fall. It has also been shown by MEYER (3) that the sugar content of the expressed cell sap of the pitch pine increases during the autumn months, is relatively high during the winter, decreases in the spring, and is relatively low in the summer.

Since there is a relation between osmotic pressure and sugar content of expressed cell sap, and as sugars have a considerable influence on the pH value of solutions, it was thought desirable to measure the pH values of expressed cell sap of *Pinus ponderosa*, and determine whether any relation exists between the osmotic pressure and the pH values of the cell sap of this tree over an extended period of time.

Method

Branches were collected in the early morning from Cedar Mountain, which is about 9 miles distant from the University of Idaho campus. The needles were picked from the branches and dust particles removed as completely as possible by the use of soil sieves and cheesecloth. The leaves were then ground in a meat chopper, using the fine knife, and the cell sap expressed by the method previously evolved (1). Very little time elapsed between collecting the leaves and the osmotic pressure and pH determinations.

The freezing point depressions were determined by use of a Beckmann thermometer, and the osmotic pressure was computed by using the following formula:

$12.05\ (T-T')$ = atmospheres of osmotic pressure
T' = freezing point of distilled water
T = freezing point of cell sap
12.05 is the ratio of the osmotic pressure, expressed in atmospheres, to the freezing point depression, in degrees centigrade, of a solution of a normal solute in water

The pH values were determined electrometrically in a Bailey hydrogen electrode, measured against a saturated calomel cell by means of a Leeds and Northrup potentiometer. The e.m.f. values so obtained were converted into the corresponding pH values from an unpublished table computed by Professor L. C. Cady of the Chemistry Department.

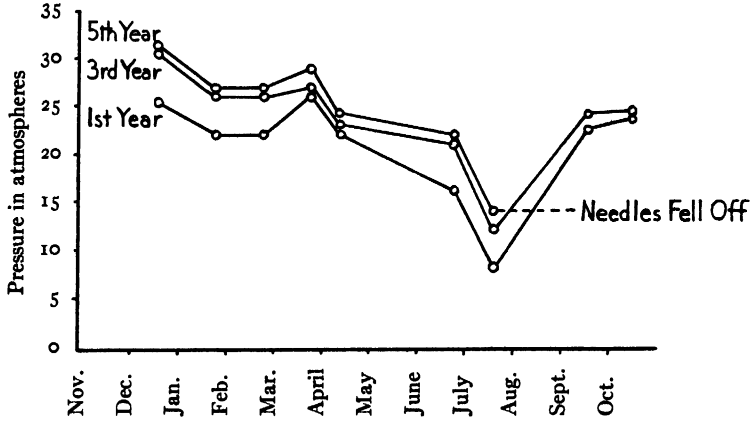

Fig 1

Experimentation

Work was begun in November, 1925, and measurements were made once a month for a year, and at irregular intervals thereafter for another year. Determinations were made on the first, third, and fifth year needles. It was noted at once that the osmotic pressure and pH values were greater in the older needles, and this was found to be true without a single exception throughout the entire period of experimentation. The first osmotic pressure measurements were made December 28, at which time there was a maximum value for the year (fig. 1). A minimum pH (fig. 2) was obtained the latter part of January. From that time there was an increase in pH until a maximum was reached in April. After this date the pH was practically constant until in July, with the exception of some variation in the first year needles. After July the pH values decreased gradually until November, when regular determinations ceased. A minimum osmotic pressure was reached during July when the cell sap still showed a high pH. The osmotic pressure increased from this time until the last measurements were made on these needles in November. The fifth year needles fell during the month of August until it was impossible to collect enough for a determination (figs. 1, 2).

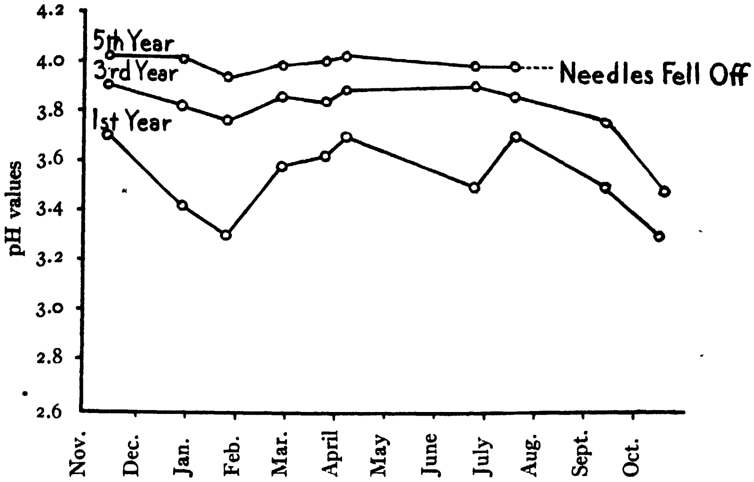

FIG. 2

TABLE I

SUMMARY OF OSMOTIC PRESSURE VALUES

AGE OF NEEDLES	SPRING TIPS	FIRST YEAR	THIRD YEAR	FIFTH YEAR
Average value for one year	3.04*	22.15	24.13	24.46
Maximum value		26.99	30.42	30.54
Minimum value for one year		7.23	11.57	12.05
Variation during one year		19.76	18.85	18.49

* Average for spring tips represents average of monthly values from time of appearance in June until in September.

A consideration of the osmotic pressure values (table I) shows an increase with the age of the needles and a decrease in the annual variation.

While there is not a direct relation between the osmotic pressures, as determined by freezing point depressions, and pH values, a summary (table II) shows an increase in pH with increase in the age of the needles and a decrease in the annual variation.

The lower osmotic pressures and the higher pH values of April, May, and June may be explained partially on the basis of rapid growth during these months. The carbohydrates and the other substances are used for growth of the new leaves and stems, resulting in a decrease of osmotic pressures.

TABLE II

SUMMARY OF PH VALUES

AGE OF NEEDLES	SPRING TIPS	FIRST YEAR	THIRD YEAR	FIFTH YEAR
Average values for one year	2.986*	3.525	3.635	3.991
Maximum value		3.702	3.888	4.142
Minimum value		3.195	3.466	3 753
Annual variation		.507	.422	.389

* Average for spring tips represents average of monthly values from time of appearance in June until in September.

It has been shown by LEWIS, MERRIAM, and MORAN (2) that the presence of sugars decreases the pH values. The substances including sugars made by photosynthesis, as well as stored materials being used for growth decreasing the sugar content, may in part account for the higher pH values during the months of April, May, and June. Growth largely ceases at this time, resulting in storage of starches and other substances. These may be changed to sugars, etc., resulting in increased osmotic pressures and decreased pH values during the winter months.

Just prior to the falling of the fifth year needles the pH was approximately 4.1. This was about the maximum value ever found in a great number of determinations covering a period of two years. Unpublished data on *Pseudotsuga taxifolia* also show 4.1 to be about the highest pH obtained in that species. This may possibly indicate that at such a pH value the enzyme action is inhibited to such an extent that life processes are no longer possible.

Summary

1. Osmotic pressure measurements made by means of freezing point depressions, and pH values determined electrometrically were made on first, third, and fifth year needles of *Pinus ponderosa.*

2. There is a high pH and low osmotic pressure during the period of growth, and a lower pH and higher osmotic pressure during the dormant period.

3. The pH and osmotic pressure of the cell sap increase with the age of the needles for any given time.

4. The fluctuation of osmotic pressures and pH values becomes less as the age of the needles increases.

5. The needles fell when the pH was approximately 4.1.

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MOTILE SPORES OF PEARSONIELLA

EARLE AUGUSTUS SPESSARD

(WITH TWENTY-SEVEN FIGURES)

The genus *Pearsoniella* was founded by FRITSCH and RICH[1] from material collected in South Africa. They state: "It is essentially characterized by its chloroplasts, which assume a form, as far as we are aware, not yet recorded among the algae, viz. that of a complete ring or girdle encircling the periphery of the cell." A letter recently received from the senior author expresses the opinion that the genus may have to be discarded after a complete renovation of the genus *Ulothrix* has been effected. PRINTZ[2] states: "Mir scheint es wahrscheinlicher, dasz sie nur eine Sektion von *Ulothrix* darstellt."

Since little is known about the method of reproduction in the genus, the following account may serve as an aid to the determination of its taxonomic position.

MATERIAL

All the material on which this account is based was raised in the laboratory from a few young plants found in water gathered at a quiet spot in the Ouachita River at Arkadelphia, Arkansas, August, 1928. Within a few days the material fruited, as shown in figs. 24–27. This type of reproduction has been seen but once since that time.

On February 7 it was observed that these spores were motile and that one was produced in a cell. They germinated in the inclosure of the outer walls in case they did not escape. The cross walls were broken down, so that as many as eight appeared in a single inclosure. By October, some of the original filaments had enlarged to many-celled filaments (fig. 1). Such filaments, when placed in fresh cultures, disappeared, and young filaments were found on the sides of the container. Material from one-celled sporelings to filaments 200 μ in diameter have been grown within five months under laboratory conditions. Plants were observed in the living condition, by microphotographs, and after sectioning. All cytological details will have to be omitted until a thorough study of the material can be made.

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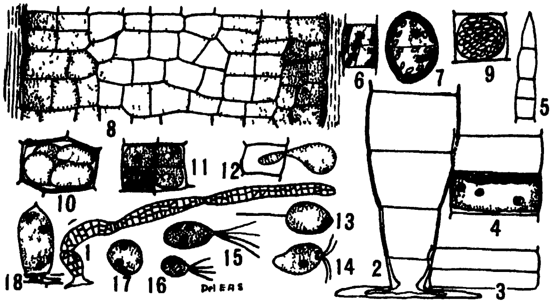
[1] FRITSCH, F. E., and RICH, FLORENCE, Contribution to our knowledge of the freshwater algae of Africa. IV. Freshwater and subaerial algae from Natal. Trans. Roy. Soc. So. Africa 11:314–317. 1924.

[2] PRINTZ, H., Ulotrichaceae, in ENGLER and PRANTL's Die natürlichen Pflanzenfamilien. 3:157–165. 1927.

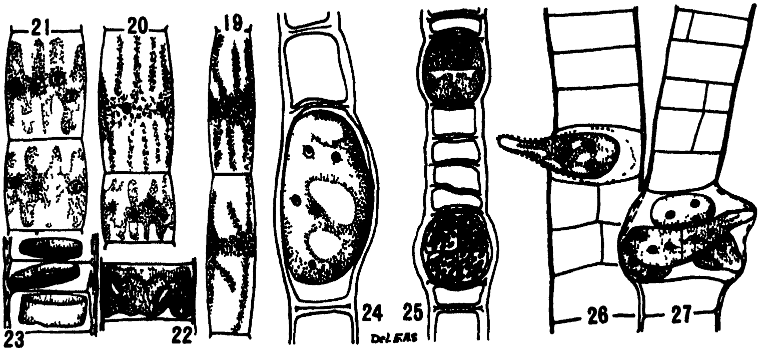


FIGS. 1–18.—Fig. 1, medium-sized filament of third phase of *Pearsoniella*. Figs. 2–5, portions of single filament from base to tip, at levels 5, 10, and 20 mm. from base; filament much narrowed at tip and widest below middle; lobed chromatophores shown in fig. 19 are present in apical or near apical cells of this filament. Figs. 6, 7, longitudinal and cross-section of filaments in second phase of longitudinal division. Figs. 9–16, stages in development and life of zoospores. Figs. 17, 18, stages in development of young sporeling; ×256 except fig. 1, which is ×55.

VEGETATIVE GROWTH

There are three comparatively distinct phases to the life history of *Pearsoniella* so far as observed. All of the stages appear to be vegetative. The first phase begins with the zoospore (figs. 12–16). This lasts during its motility, for half an hour to an hour. This short motile period is rather unusual; in *Ulothrix* the time is often 24 hours. The short period may be caused by the excessive light under the microscope, or possibly the heat. The second phase (figs. 2–5, 17–23) is the sporeling and elongation phase. The filament reaches a length of 2 cm. within 10 days, without any longitudinal division of the cells. Sometimes, however, longitudinal division begins in isolated cells before this length is reached. The third phase is initiated by longitudinal division of the cells. It continues until the filament attains a diameter of 200 μ. Zoospores are produced during the second and at the end of the third phase.

Mitotic figures have not been observed; consequently it is not safe to state precisely what is the nature of wall formation in the large filaments. It is not certain whether the filament is a hollow cylinder or a solid mass of cells. Sectioned material shows that there is a hollow space in the middle of some of the largest filaments of the third phase, nearing the production of zoospores. This point is being investigated. Fig. 7 shows a cross wall in a filament two cells wide. In fig. 6 is shown a longitudinal wall in a filament of the third stage at the beginning of longitudinal division. In some sections there appears to be distinct apical activity, but these are isolated cases and cannot be used as evidence. From the outside appearance of the largest filaments, as well as the smallest, walls appear to show distinct relationship to the original divisions. In fig. 5 the third cell has divided without changing the contour of the mother cell In fig. 1, the many-celled filament of the third phase shows constrictions that suggest a zonal activity coordinate with the original cells of elongation like those shown in figs. 2–5. The filaments reach a length of at least 6 cm. and a width of 60 μ by the time the longitudinal walls become general.



FIGS. 19–27.—Figs. 19–23: band-shaped chromatophores in older and basal cells in figs. 22 and 23, as well as band-shaped and deeply lobed chromatophores characteristic of cells of second phase of vegetative growth; ×452. Figs. 24, 25: portion of two filaments showing stages in development of zoospores from young filament of third phase; possibly representing abnormality; ×256. Figs. 26, 27: germination and escape of zoosporelings in early stages of third phase; ×452.

The filaments of the third phase twist and bend about to form a tangled mass 3 cm. in diameter. The size, of course, depends upon the number of threads present originally. From the culture records of sporelings observed, there is apparently no period of rest. One mass of filaments in the third phase was cut in half in November and it doubled its size by January 31. During this time two sets of sporelings were started from spores approximately 20 days apart. It appears that spores are produced periodically, and that the cells remaining in the large filament continue to vegetate.

ZOOSPORE PRODUCTION

Zoospores have been observed to discharge on ten different occasions. On each occasion they were produced by filaments of the third phase. The first discharge occurred in material contaminated with other algae; the second discharge yielded but one zoospore; the third occurred at noon, and several hundred spores were observed from the moment of discharge until they settled down. Previous to these discharges sporelings of all stages had been seen.

Fig. 12 shows a zoospore emerging from the cell of a large filament. The passage out is always taken suddenly and singly. The mother cell shown in the drawing is semidiagrammatic, but living material shows that the passage is as shown in the figure. In some instances a long fine line (fig. 13) can be seen to hang on to the spore a few minutes after discharge. In one instance a small green spherical mass remained attached to this thread, and behind this a second irregular mass of protoplasm, starch, and chromatophores, four times the volume of the spore, was attached. The spore thus encumbered continued to drag its load, tandom fashion, from one side of the slide to the other.

Surface views (figs. 10, 11) leave some doubt as to the number of spores in each mother cell; sections no doubt will explain this point. In filaments as large as these it is impossible to observe whether spores discharging near one another, and very close to one another in time, really come out from one cell containing four or from one containing one. Fig. 8 shows the surface view of the filament from which the zoospores figured were discharged. The shading does not indicate the contents. In fig. 10 there is shown one cell with four zoospores. No membranes could be seen. Fig. 11 shows four cross walls, but these may be separate from the wall proper at discharge. The tail-like affair shown in fig. 13 seems to be explained only on the assumption that a membranous sheath escapes with the zoospore.

ZOOSPORE

The zoospore possesses four cilia slightly longer than the body. There are two contractile vacuoles situated in the clear region at the base of the cilia, and a short pointed beak from which the cilia arise. A red stigma is always present. The average diameter is 14 μ, and the average length 25 μ.

Fig. 16 shows a type of zoospore 9×15 μ. These spores have been seen to settle down and produce filaments. No instance of fertilization has yet been observed in this material. The exact nature of the spores shown in figs. 24–27 is not determined. They are motile, and in all healthy material observed only one to a cell was produced. In what respect the two types of spores differ is not yet determined.

From this brief account, *Pearsoniella* appears to have a much longer life history than has hitherto been supposed. The presence of the large zoospore-forming filaments will necessitate some slight changes in the original description. This the writer of course leaves to the judgment of the original authors, to whom material is being sent for inspection.

Summary

1. *Pearsoniella* is reported for Arkansas.

2. The mature vegetative stage is an immense, many-celled cylinder possessing a vegetative growth of at least two months.

3. The zoospores are produced at two stages in the life history, the early and late stages of the third phase, and last stages of the second phase.

4. Heterospory or heterogamy is suggested by the observation of unlike "zoospores."

5. No fertilization was observed.

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BRIEFER ARTICLES

A HEAD OF SORGHUM WITH GREATLY PROLIFERATED SPIKELETS[1]

(WITH THREE FIGURES)

An unusual head of sorghum was found in a field of Blackhull kafir by D. L. ROSER, Burlington, Coffey County, Kansas, October 19, 1928. It was taken from a bundle at the time the crop was harvested and no other specimen of this kind was observed. The field was practically pure and nearly free of smut. The seed was reported to have been grown in Coffey County, and was obtained from the Burlington Feed and Seed Store. Blackhull kafir is an adapted and extensively grown variety in the vicinity of Burlington.

The panicle (fig. 1) was about the size and shape of a normal head. It was late in maturing, and contrary to the usual habit the spikelets at the tip of the panicle were last to reach full development.

Examination of a number of spikelets revealed the normal paired condition of the genus *Sorghum*. The upper spikelet was always small, about 3.5–4 mm. long, slender, villous on the pedicel at the juncture of the spikelet, and finely appressed hairy outside. It usually was made up of four scales (3–5), all of which were lanceolate, hyaline-margined, and sparingly appressed pubescent. No trace of stamens or pistils was discovered. The lower spikelet, however, was very curiously modified. Instead of the normally fertile condition, a series of scales extended upward from between the glumes (figs. 2, 3). A glance suggested a spikelet of an *Eragrostis*. No trace of grain or of grain-producing structures was found on any specimen examined. The spikelets varied in length from 3.5 to 10 mm. (usually about 9 mm. when fully expanded), and in width from 4 to 5.5 mm. (usually about 5.2 mm.). They were conspicuously villous at the juncture with the pedicel, in the same manner as the upper spikelets. When fresh, or later when soaked, these spikelets were the shape of a cone flattened at right angles to the scales, but when dried they were more or less oblong, except for the slight taper near the outer part and a prominent shallow groove running nearly the full length of the flattened sides.

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[1] Contribution no. 181, Department of Agronomy and no. 292, Department of Botany and Plant Pathology, Kansas State Agricultural College, Manhattan, Kansas.

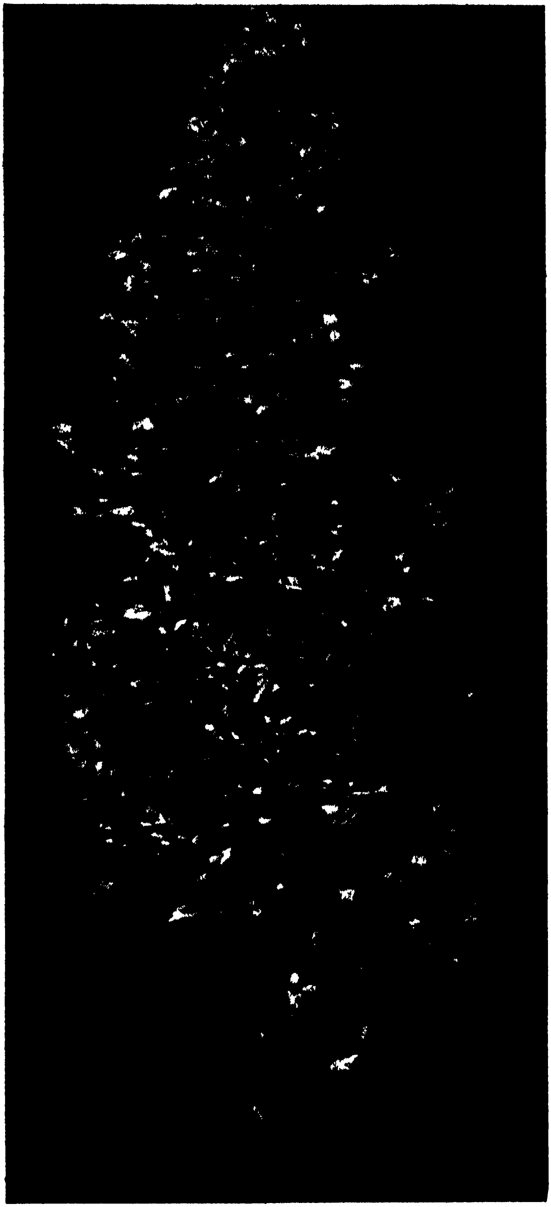

FIG. 1.—Unusual panicle of Blackhull kafir; about 1/3 natural size

The glumes were well developed, coriaceous, divergent, straw yellow, broadly ovate, and well rounded on the back, conspicuously hyaline and sparingly villous with projecting white hairs and slightly pubescent with appressed hairs. Above the glumes were about 38 (28–41) scales inserted closely, each ovate, or toward the tip of the spikelet ovate-lanceolate, well rounded on the back, bluntly or somewhat sharply pointed, but never awned, with 5 or 7 broad principal veins running obviously from near the tip. When magnified these appeared double, with two green stripes on either side of a white stripe. Between these veins there was usually an additional vein which stopped short of the others, but which was connected with them by veins running at right angles. In the soaked scale the vein network was observed to form a conspicuous pattern, which in the dry scales was obscured by the silky hairs that were particularly abundant toward the edge of the scale. Beyond the outermost veins was

FIG. 2.—Cluster of spikelets showing unusual modification (divisions of scale below are millimeters).

a rather broad hyaline margin which infrequently was in part a deep red color. This color occurred more frequently on the lower or middle scales, but was not sufficiently abundant to be a conspicuous point.—H. H. Laude and F. C. Gates, *Kansas State Agricultural College, Manhattan, Kan.*

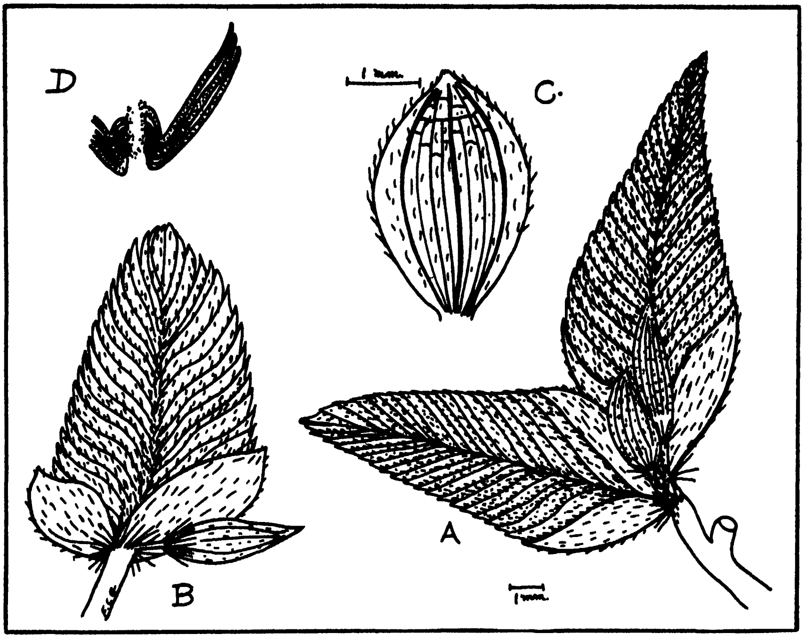

Fig. 3.—Diagram illustrating details of spikelets: *A*, fresh spikelets; *B*, spikelet thoroughly dried; *C*, single scale (lemma) showing peculiar venation; *D*, insertion of scales.

[*Accepted for publication February 11, 1929*]

CURRENT LITERATURE

BOOK REVIEWS

Plant sociology

The trend of ecological thought in Europe has always differed somewhat from that which is at the time in vogue in America. It is peculiarly important, therefore, that American ecologists keep as close an understanding as possible of what is going on across the water. For this purpose the little volume by BRAUN-BLANQUET[1] is most admirable. His citation of literature is extensive, and extends right up to the date of publication. The very active Scandinavian and Russian ecologists are freely quoted, and much American research is reviewed. The book is a detailed summary of research methods, in fact, together with a critical evaluation of each proposal, and much sound philosophizing.

The introductory page points out the difference between studies of the individual organism and studies of communities. Choosing for this volume the second viewpoint, plant sociology is defined as the study of all the phenomena that have to do with the life of plants in social units. Section I discusses commensalism very briefly, and competition at length. Section II, the remainder of the book, presents in order the five principal problems of plant sociology: (1) organization or structure of communities; (2) synecology, the relation of communities to one another and to the rest of the environment; (3) syngenetics, the origin and decline of plant communities; (4) synchorology, the occurrence and distribution of communities; (5) classification of communities (systematics).

The first topic (organization) stands today at the focus of phytosociologic activities. The structure of the community "is the indispensable foundation for an unbiased treatment" of all the related problems. And it is this section that is most needed in American thought and practice. A difficulty arises in finding English equivalents for the terms Assoziation, Einzelbestand, Subassoziation, Fazies, Assoziationsfragment, Siedlung, Vegetationsfleck, Verein, Synusie, and Formation. It is indeed apparent that formation, association, society, as proposed by CLEMENTS and by LIVINGSTON and SHREVE, do not exactly correspond with the categories used by European botanists. It is to be hoped that a clear definition of terms may be decided upon at the forthcoming Cambridge conference.

The characteristics of a community are considered under the following heads:

1. Analytic characteristics.—

(a) Quantitative: number of individuals (abundance) and density; covergrade, volume and weight (dominance); grouping (sociability) and distribution; frequence.

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[1] BRAUN-BLANQUET, J., Pflanzensoziologie. pp. x+330. *figs. 168.* Berlin: J. Springer. 1928.

(b) Qualitative: layering; vitality (health); periodicity.

2. Synthetic characteristics.—
   (a) Constancy.
   (b) Fidelity.

These concepts are clearly defined, and procedure is given for their determination. The result is shown by an example. Such procedure permits accurate comparison of data taken in any kind of vegetation in any part of the world.

Synecology treats in the usual way of heat, light, water, and wind. The section on soil is much more complete than in any other such text known to the reviewer, and is full of excellent data and constructive criticism. Life-forms are treated in the latter part of this section.

Syngenetics has to do with the up-building of communities, and includes the study of succession. About twelve pages suffice to outline this topic. The chapter ends with recent geological stages and pollen analysis.

Synchorology deals with the geographic extent and distribution of communities, both in breadth and altitude. Zonation is discussed here. Pioneer and relict communities are considered. The surface of the earth from the standpoint of vegetation is divided under the headings Region, Provinz, Sektor, Bezirk, District, Unter-district (Gau).

The classification of communities, "like every natural classification, presupposes the most exact knowledge of all parts of the problem. It follows from the nature of the case that this portion of the science of communities, lacking as yet the broad foundations that are necessary, is at present the least developed." Proposed systems of classification are cited and criticized. The last two pages offer a classification of communities based upon their sociological progression from simpler to more complex structures. Without attempting to be syngenetic or successional in arrangement, the series begins with the indefinite floating vegetation of air and ocean with few species (bacteria, algae, fungi), and culminates in the many-layered forest community with a multiplicity of species. It has the merit of simplicity, and it seems to provide a place for every kind of natural grouping of plants.

BRAUN-BLANQUET has given us in this work an ecological guidebook of first importance. The volume is crammed with ideas, methods, and results. The author has a fondness for inverted sentences which are somewhat difficult to the Anglo-Saxon mind; but his style is forceful, and his treatment analytic and fair. His examples bring out every point and process with clearness. The halftone illustrations of natural vegetation are really beautiful, and to the point. The book seems to be without a mechanical flaw.—H. S. CONARD.

Plant ecology

More than thirty years ago two centers of ecological study and teaching developed simultaneously in America, at the Universities of Chicago and Nebraska, and both schools have since contributed largely to the development of this branch of plant science, agreeing in placing great emphasis on the dynamics of vegetation as expressed in plant succession. Both schools have been more interested in the investigation of the problems of vegetation than in the production of textbooks.

As the years have passed, however, and as some order has emerged from the intricacies of the relations between the plant, or the plant community, and its environment, the need of at least one comprehensive text on plant ecology has been felt. This demand has now been supplied from one of the original centers.[2] It seems particularly fitting that this book should have been written by Nebraska's pioneer ecologist, CLEMENTS, and one of his students, WEAVER, the latter for many years a successful investigator and teacher.

Perhaps the first impression gained by glancing through the 500 pages of this volume is the diversity of the topics discussed. Nor is this diversity in topics surprising when the complexity of the plant environment is recognized, and the varied applications now being made of the results of ecological investigations appreciated. An effort has been made, and a very successful effort, to discuss all these topics in a somewhat exhaustive manner. Among the portions of the text that seem to the reviewer most satisfactory, are the chapters or sections dealing with plant succession, competition and invasion, methods of studying vegetation, structure and ecology of roots, measurement and effects of evaporation and temperature, plant indicators, and the climax formations of North America. A map, which is remarkable for its simplicity, gives the limits of these formations. The units of vegetation, soil formation and soil structure, and the adaptations of plant structures seem to be less satisfactorily discussed.

With the great advances made in soil science during the past two decades, it is quite impossible to discuss the subject adequately within the limits of a dozen pages, nor is it surprising that an attempt to do so is rather unsatisfactory. It is noted, for example, that the difference between the hygroscopic coefficient and the wilting coefficient of soils is not clearly expressed. It is made clear that each is a function of the texture of the soil as related to its water content, but of each it is said that it represents the amount of water remaining in the soil when permanent wilting of plants occurs, and yet the wilting coefficient is nearly twice as great as the hygroscopic coefficient.

The units of vegetation are still burdened by an excessive terminology, a fault that seems evident in other portions of the book.

The rapid advance now being made in certain branches of plant ecology is nowhere more clearly shown than by a comparison of the chapter on leaf structure or "adaptation to water" with a corresponding chapter in MAXIMOV's[3] book, which appeared only a few weeks later than the American text. We believe that WEAVER and CLEMENTS, together with most ecologists, will be convinced by the experimental data presented by MAXIMOV that such xeromorphic structures as greater thickness of leaves, more palisade tissue, more conductive and mechanical tissue, smaller epidermal cells (that are more highly cutinized and are accompanied by smaller but more numerous stomata), result in an increase and not in a decrease in transpiration. It is true, however, that leaves with such xeromorphic structures are more drought resistant in spite of their higher transpiration.

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[2] WEAVER, J. E., and CLEMENTS, F. E., Plant ecology. 8vo. pp. xx+520. *figs. 262; colored map.* New York: McGraw-Hill Book Co. 1929. $5.

[3] MAXIMOV, N. A., The plant in relation to water. London: George Allen & Unwin Ltd. 1929.

Notwithstanding such slight and largely unavoidable deficiencies, and certain minor errors which it would be invidious to enumerate, the authors are to be congratulated upon the successful outcome of their efforts. They have presented a multitude of data in a logical and well organized manner. Their discussion is broad and convincing; their citations show a thorough acquaintance with the latest as well as with the older literature. There is an occasional leaning toward teleological interpretation, but on the whole there is continued evidence of scientific accuracy. At times, however, the authors speak with a definiteness and conviction that most students of ecology find it impossible to share.

This volume may not commend itself to those who wish to teach a textbook; but for teachers and students really interested in science it will be a standard text for many years. While its organization may not exactly fit the needs of any particular class, it abounds in excellent suggestions for laboratory and field exercises, and will be found an indispensable aid in interpreting the innumerable phenomena of vegetation. The well chosen illustrations and the extensive bibliography also help in making it by far the best and most comprehensive textbook on the subject that has yet appeared.

The printers and publishers have done their share in producing a book which it will be difficult to excel.—G. D. FULLER.

Plant rusts

The fields of mycology and phytopathology are so vast that one person cannot keep abreast of all their aspects. Consequently, the volume on the plant rusts by ARTHUR[4] and his students and associates is a most welcome and timely addition to mycological and phytopathological literature, by a group of competent specialists. It is hoped that their example will be followed, and that separate comprehensive treatises on other biologically interesting groups of fungi, such as the Ustilaginales, for example, will appear in the near future. Such volumes should summarize what is accepted as established fact and established concept, point out what is problematical, and suggest working hypotheses to fill in the gaps and to stimulate new investigations. The volume under discussion in great measure lives up to these demands. It is characterized by comprehensiveness, clarity of expression, and an interesting, easy style. It contains chapters on: general nature of the rusts; historical review; development and classification; cytology and morphology; dissemination and geographic distribution; physiology; specialization; teratology and pathology; ecological considerations; methods of investigation; and a comprehensive bibliography. The value of the volume would have been greatly enhanced if a key and list of known rusts had been included.

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[4] ARTHUR, J. C., in collaboration with KERN, F. D., ORTON, C. R., FROMME, F. D., JACKSON, H. S., MAINS, E. B., and BISBY, G. R., The plant rusts (Uredinales). pp. v+446. *figs. 186.* John Wiley and Sons. 1929.

It is stated (page 2) that the terms gametophytic or haploid phase, and sporophytic or diploid phase are not strictly applicable to the rusts. Certainly the terms gametophytic and sporophytic are not at all applicable, especially the former; but it does not follow, therefore, that the terms haplophase and diplophase are not applicable. In fact, their use would make for precision and clarity. Really to express the situation one should state that the life cycle of a "typical" rust is composed of three phases, the haplophase, the dicaryophase, and the diplophase. It is unfortunate that the authors have used the concepts and terms gametophytic and sporophytic as the framework of a great deal of their presentation.

At the beginning of chapter III the fundamental features of rusts are stated quite canonically. The following statement occurs: "the vegetative body is macrocyclic (long cycle), consisting of two unlike and discontinuous generations." Later in a discussion which justly stresses the importance of the mycelium it is stated "the aecia are initiated by the gametophytic mycelium." It may develop that these statements really represent the facts, when rust pustules are reinvestigated in the light of recent reports by CRAIGIE, HANNA, and ALLEN, but one is justified in hazarding the guess that more cases will be found of more or less extensive continuous development of dicaryomycelium from one or two haplomycelia, and of organization of aecia by either the haplomycelium and the dicaryomycelium, or by the dicaryomycelium alone. Of course, one can ask, When is an aecium an aecium? Should differentiation of the structures described by CRAIGIE, HANNA, and ALLEN in monosporidial pustules be considered as initiation of aecia? It is of interest that Miss ALLEN, in referring to these structures, uses the guarded phrase "structures resembling aecia." Although many guarded statements occur relative to sexuality, a definite stand is taken in chapter III for sexuality on the insecure ground that fusion or union occurs between hyphae, and eventually between nuclei.

One has the feeling that much needless effort was spent in harmonizing divergent views of the various contributors. (It is stated in the preface that "there remain here and there occasional statements which are not endorsed by one or more of the several authors.") Possibly the volume would have been even more stimulating than it is, if at the cost of no more than artificial harmony and unity, each contributor had been given free rein to express his own ideas and assume full responsibility for signed chapters.

It is a timely volume, admirably summarizing the present state of knowledge relative to rusts. It comes at a time when genetic analysis of fungi is in the air and a new theoretical and experimental approach to the problem of specialization and of physiological races and forms is imminent, in the light of CRAIGIE's discovery of heterothallism in the Uredinales, of GOLDSCHMIDT's highly suggestive genetic analysis of specialization in *Ustilago violacea*, and of DODGE's interesting study of hybridization with *Neurospora*.—G. K. K. LINK.

Insects

Three volumes on insects have appeared which are of general biological interest, and lend themselves to use by anyone interested in plants and their enemies. The volume by METCALF and FLINT admirably covers its chosen field.[5]

The first chapters are devoted to a general consideration of insects, such as their importance to man and domestic animals, and their structures and life histories. These are followed by a presentation of the orders of insects, and a discussion of control measures. The remainder of the major portion of the book is devoted to a special part in which crops form the basis of discussion of insects which parasitize them. The volume is a signal contribution in the field of entomology.

The volume by GRAHAM,[6] although it is independent and is built along different lines, forms a companion book to the former, in that it takes up forest diseases, which are not considered by METCALF and FLINT. Because of the great importance of insects in the forest and in relation to timber decay by fungi, this volume will be welcomed by phytopathologists. The first four chapters are devoted to general biologic considerations relative to insects. Chapters VII–XIX are devoted to general control measures; chapters X–XV take up insects on the basis of the tree tissues attacked by them; and chapters XVI and XVII discuss the parasites of forest insects.

The volume by WARDLE[7] is evidence that entomology is ceasing to be a congeries of isolated facts about individual insects, with a casual glance at the host they infest, and is striving to be a real science. The first part of this interesting volume, which is permeated by a theoretical atmosphere, is devoted to a consideration of general problems such as host resistance, theory of insecticides, and cultural influences. The second part concerns itself with area problems, in which in addition to general theoretical considerations, the material for each major geographic area is presented so that the non-resident may form some idea of the problems which occur in that area. This is of great use in connection with quarantine problems. There is an excellent bibliography.—G. K. K. LINK.

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[5] METCALF, C. L., and FLINT, W. P., Destructive and useful insects. pp. xii+918. *figs. 561*. New York: McGraw-Hill Book Co. 1928.

[6] GRAHAM, S. A., Principles of forest entomology. pp. xiv+339. *figs. 149*. New York: McGraw-Hill Book Co. 1929.

[7] WARDLE, R. A., The problems of applied entomology. pp. xii+587. *figs. 31*. New York: McGraw-Hill Book Co. 1929.

Flora photographica

A very significant and comprehensive study of the vegetation of the world has just been initiated in a volume[8] of which Dr. HUGO ILTIS of Brunn is the editor. This book has for its main object the publishing of actual photographic prints, depicting the chief vegetative features of the entire world, country by country. The first volume to appear is volume II, which deals with the floral province of the European "Mittelgebirge." In the preface it is noted that this work is designed not only for botanists, but also for geologists, agriculturists, and friends of the plant world. The photographs are to be of uniform size, 9×12 cm., and it is hoped that this work will prove a notable complement to herbaria and floras. The publishing of photographic prints rather than the reproductions from these prints is highly advantageous, because none of the details of the picture are lost. It is proposed to publish two or three numbers annually, either in centuries or half centuries. In each volume a certain floristic area will be dealt with, on the basis of ENGLER'S classification. The work is published in three editions, German, French, and English. The volume under review was translated by Mr. W. C. WORSDELL. It has a complete index of plant names. In this first century the photographs are grouped in twenty-five different formations, some of which are the *Pinus silvestris* forest, birch forest, spruce forest, mixed mountain forest, beech forest, hornbeam forest, lowland and riverside forest, forest clearing, the copse and forest-edge, heath, rock, dry mountain-meadow, valley and riverside meadow, and moorland meadow. If this first volume to appear is any indication of what is to follow, we cannot have the later numbers too soon. In the opinion of the reviewer, no such great and concise treatise of the vegetation of the world, area by area, has been presented in a form so easily understandable.—H. C. COWLES.

NOTES FOR STUDENTS

Anthocyanins.—The anthocyanins have long been of interest to botanists as natural indicator pigments. Recently FEAR and NIERENSTEIN[9] have pointed out that the color reactions of these compounds should be examined in solutions of definite pH, if such studies are to be valuable in comparison and characterization of the various anthocyanins and their derivatives. It is now claimed by ROBERTSON and ROBINSON[10] that examination of the reactions of the anthocyanidins in a range of buffered solutions is by far the most reliable method for such comparisons, even of greater usefulness than the study of absorption spectra, for some different anthocyanidins (as peonidin and syringidin), as demonstrated by SCHOU,[11] show identical absorption sepectra, but can easily be differentiated by their reactions in the presence of buffered solutions. At the same time, the buffer method of examination reveals such phenomena as pseudo-base formation, color-base precipitation, and the ease of oxidation of the compounds.

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[8] ILTIS, H., and SCHULZ, B., Flora Photographica. II. Floral province of the European "Mittelgebirge" I. Transl. by W. C. WORSDELL. pp. 50. *pls. 100.* Brunn: Rudolf M. Rohrer. 1928.

[9] FEAR, C. M., and NIERENSTEIN, M., The colour variations of cyanidin chloride and 3:5:7:3′:4′-pentahydroxyflavylium chloride as related to acidity and alkalinity. Biochem. Jour. 22:615–616. 1928.

[10] ROBERTSON, A., and ROBINSON, R., Note on the characterization of the anthocyanins and anthocyanidins by means of their colour reactions in alkaline solutions. Biochem. Jour. 23:35–40. 1929.

ROBERTSON and ROBINSON do not agree with FEAR and NIERENSTEIN that cyanidin chloride differs from synthetic 3:5:7:3′:4′-pentahydroxyflavylium chloride, but emphatically insist that the pure synthetic compound, prepared from its benzol derivative by hydrolysis, exhibits no differences, even to the minutest details, from the natural cyanidin chloride. Claiming that the structural constitution of the carbon-oxygen skeleton of cyanidin chloride is as firmly established as that of any other compound, they reject the attempt of MALKIN and NIERENSTEIN[12] to modify the cyanidin formula to reconcile some earlier and conflicting studies in catechin chemistry.

The series of buffers used by ROBERTSON and ROBINSON were made from phenyl-acetic acid, boric acid, and KH_2PO_4, 0.02 gm. each, with additions of different amounts of 0.2 N NaOH, dissolved in water and made up to 1000 cc. The range in seventeen steps was from pH 3.2 to approximately pH 13. The reactions of various anthocyanidin chlorides are described after standing 24 hours in the buffers. They include the chlorides, apigeninidin, pelargonidin, cyanidin, peonidin, and malvidin. The reactions of the chlorides of two anthocyanins, cyanin and malvin, are also described. The changes with changing pH and with time are distinctive, and the method should prove valuable in grouping the many naturally occurring but yet unstudied anthocyanins.—C. A. SHULL.

Taxonomic note.—Another posthumous part of the late J. H. MAIDEN'S monumental revision of *Eucalyptus*[13] is at hand. No new species and in fact only two new varieties are described. There is appended a digest of opinions from various sources as to "the species question." These bear upon such topics as: what is a species, variety or species, inequality of species values, no fixed line of demarcation between species, JORDAN'S species, classical case of splitting, application of zoological tests to a botanical species, and variation in the genus. They are followed by a section on the struggle for taxonomic definiteness, in which are given discussions of the ideal of the type, how to designate the type, model descriptions, and labels and schedules; and finally, a list of pertinent aphorisms taken from DARWIN, HUXLEY, and others.—E. E. SHERFF.

[11] SCHOU, S. A., Über die Lichtabsorption einiger Anthocyanidine. Helv. Chim. Act. 10:907–915. 1927.

[12] MALKIN, T., and NIERENSTEIN, M., Zur Kenntnis des Cyanidins. I. Vergleichende Untersuchungen über Cyanidinchlorid und 3.5.7.3′.4′-Pentaoxy-flavyliumchlorid. Ber. Deutsch. Chem. Ges. 61:791–799. 1928.

[13] MAIDEN, J. H., A critical revision of the genus *Eucalyptus*. 7:405–450. 1928.

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